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SIXTH SCIENTIFIC REPORT

ON THE INVESTIGATIONS

OF

THE IMPERIAL CANCER RESEARCH FUND.

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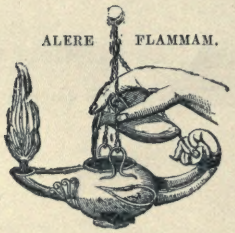

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CONTENTS.

	Page
PREFACE	vii
OBSERVATIONS ON THE FUNCTIONAL ACTIVITY OF THE SUPRARENAL GLAND IN HEALTH, AND IN DISEASE. By W. CRAMER. (Plates I.–V.)	1
Introduction: The function of the thyroid-adrenal apparatus	1
The histo-chemical method	2
The normal "resting" gland	3
Response of the gland to powerful stimulation: Lethal doses of tetrahydronaphthylamine, severe exposure to cold	5
Response of the gland to a slight stimulation: Exposure to cold of moderate degree	7
Self-regulation of the "load" of adrenalin	8
Depletion of the load of adrenalin	8
Changes in the cortex accompanying activity of the medulla	9
Significance of the "load" of adrenalin	10
Effect of acidosis, hæmorrhage, and anæsthesia	11
Effect of bacterial infection: gas gangrene, streptococcal abscess, streptococcal cellulitis	12
Changes in human glands in cases of acute pyrexia	13
Some changes in the thyroid gland associated with activity of the suprarenal gland	15
The suprarenal gland in post-operative shock	16
General Discussion	16
Summary	20
References	21
ON THE MECHANISM OF BACTERIAL INFECTIONS, WITH SPECIAL REFERENCE TO GAS GANGRENE. By W. E. BULLOCK and W. CRAMER. (Plates VI.–X.)	23
I. On the Lethal Mechanism of Gas Gangrene and on the importance of accessory factors in relation to it	23
Effect of exposure to cold on inoculation with broth-cultures of B. Welchii	27

Page

Effect of exposure to cold on the action of the toxin of B. Welchii 28
Effect of acidosis on the action of the toxin of B. Welchii . . 28
Effect of injection of "T.H.N." on inoculation with broth-cultures of B. Welchii 28
Effect of injection of T.H.N. on the action of the toxin of B. Welchii 29
Summary 34
References 34

II. On the Defensive Mechanism against the Bacteria of Gas Gangrene 35

III. On a New Factor in the Mechanism of Bacterial Infection . . 40
Introduction 40
Calcium Salts as an Accessory Factor in Gas Gangrene and Tetanus 42
On the Etiology of Gas Gangrene and of Tetanus 46
On the Mode of Action of Calcium Salts 49
Summary 56

IV. Further Observations on the Mechanism of Bacterial Infection. 57
Introductory 57
Lesion produced by Calcium Salts 58
Other Substances capable of producing the Phenomenon of Defence Rupture 59
The Effect on the Tissues of Substances not capable of producing Defence Rupture 61
On the Action of Lactic Acid 62
Is the Phenomenon of Defence Rupture due to the Production of Conditions favourable to Anaerobiosis? 63
Is the Presence of Muscle Tissue necessary to evoke the Pathogenic Action of the Bacteria of Gas Gangrene? . . . 64
The Phenomenon of Defence Rupture in Streptococcal Infections 65
On the early Stages of the Infection by the Bacteria of Gas Gangrene 67
General Discussion and Summary 68

Cellular Changes in Cartilage Grafts. By J. A. Murray. (Plate XI.) 71

Histological Methods of demonstrating Bacteria in Tissues. By J. A. Murray. (Plate XII.) 77

PREFACE

THE Sixth Scientific Report is published as a record of work done in the laboratories in the last four years on subjects unconnected with cancer, suggested by war conditions.

The main part of the Report is contained in the contribution by Dr. W. E. Bullock and Dr. W. Cramer "On the Mechanism of Bacterial Infections with special reference to Gas Gangrene." The first two chapters of this paper are reports by the authors to the Committee on Anaerobic Bacteria, of which Dr. Bullock is a member. The paper by Dr. Cramer on the suprarenal gland in health and disease, besides being of general importance, is necessary to full comprehension of the main paper and it is, therefore, printed first in the Report and acts as an introduction to the detailed analysis of the pathology of gas gangrene which follows.

The two remaining short communications have arisen out of the work of reporting on pathological specimens for Medical Officers at home and in France.

The photographs and microphotographs are by Mr. A. J. Hall. The drawings for the illustrations (excepting those for the paper on Cartilage grafts) have been made with the camera lucida by Mr. W. Pilgrim of the laboratory staff.

J. A. MURRAY.

[*Sixth Scientific Report of the Imperial Cancer Research Fund*, 1919.]

OBSERVATIONS ON THE FUNCTIONAL ACTIVITY OF THE SUPRARENAL GLAND IN HEALTH, AND IN DISEASE.

By W. CRAMER.

(Plates I.–V.)

Introduction: On the function of the thyroid-adrenal apparatus.

In the last four years a number of brief communications have been published from this laboratory giving a summary of experimental observations which lead to the general conclusion that the thyroid and adrenal glands functioning together form an apparatus for the heat regulation of the body by means of their internal secretions. In order to avoid misunderstanding, it may be added that this conception of the existence of a humoral mechanism is not intended to exclude that of a nervous mechanism, but rather to supplement it. The action of this humoral mechanism may be briefly reviewed as follows.

The increased secretion of the thyroid hormone leads to a mobilisation of the liver glycogen. This is brought about indirectly by the stimulating action of this hormone on the suprarenal gland, and partly perhaps also by an increased sensitiveness of the sympathetic nerve endings to adrenalin. The body reacts to the resulting influx of sugar into the blood stream by an increased oxidation, and not by a glycosuria, as the current conception of carbohydrate metabolism would lead one to expect. The heat production is thus increased. This increased heat production is not dependent on the presence of preformed carbohydrate within the food or in the liver. In the absence of such preformed carbohydrate it is formed by the liver from proteins and perhaps also from fats. The increased protein and fat catabolism which is such a well-known and outstanding feature of hyperthyroidism, whether experimental or pathological, is thus explained as a secondary effect due to the increased mobilisation of the liver glycogen. The increased heat production, which is postulated here as resulting from an influx of sugar into the blood-stream is in agreement with the recent work of Lusk, who demonstrated a considerable increase in heat production after the ingestion of dextrose.

While the increased activity of the thyroid and adrenalin apparatus is thus able to account for an increased production of heat, it is also able to control the heat loss. An increased secretion of adrenalin if sufficiently strong will contract the arterioles and thus diminish the heat loss.

The activity of the thyroid adrenal apparatus in relation to the heat regulation of the body is demonstrated very clearly in the experimental hyperpyrexia produced by the injection of tetrahydronaphthylamine. The rise in body temperature which in rats and rabbits follows the injection of this substance, and which lasts for several hours, is accompanied by an intense congestion of the thyroid gland with changes in the colloid, a very active secretion of adrenalin, disappearance of glycogen from the liver, and constriction of the superficial blood-vessels so that the skin feels cold, together with other effects of stimulation of the sympathetic system such as dilatation of the pupil and of the palpebral fissure. There is also an intense congestion of the lungs, and if too big a dose has been given the animal dies with hæmorrhages into the lung as if an overdose of adrenalin had been given. It could also be shown that with such a small animal as the mouse the dose of tetrahydronaphthylamine which could be injected without producing death, depended on the temperature of the room in which the animals were kept: mice kept in a warm room died with a dose (3 mg.) which mice kept in a cool room could tolerate.

It is intended to give a detailed account of these investigations in a separate publication. The present paper only deals with the application of this conception of the thyroid-adrenal apparatus to a number of pathological conditions on which it has obviously an important bearing, and which have become actual during the past few years. The conditions referred to are, bacterial infections associated with a pyrexia on the one hand and with a subnormal temperature on the other, exposure to cold, shock, hæmorrhage, anæsthesia, experimental hyperthyroidism produced by feeding with thyroid gland, and acidosis.

The histo-chemical method.

In these observations the functional activity of the suprarenal gland was studied by a new histo-chemical method by which it is possible to stain the fine adrenalin granules both within the cell of the resting gland and also in the intercellular spaces and blood-vessels of the actively secreting gland. The method which has already been briefly

described is as follows:—If mice are used the gland is freed as much as possible from the closely adhering fatty tissue. The entire gland is then suspended in osmic vapour at about 40° C. for 1½ hours. A simple and convenient method is to put the tissue on a piece of gauze stretched over some wide glass tubing about 1·5 cm. high, and to place the glass tubing in another small flat bottomed stoppered glass vessel containing 2 % osmic acid. After having been suspended and fixed in osmic vapour in this manner for 1½ hours, the tissue is put in 50 % alcohol, passed through alcohol in increasing strength, and embedded in paraffin in the usual way. The cut sections are mounted in balsam without further staining. If larger animals are used, thin slices of the gland must be cut with a sharp razor. With rats it is sufficient to halve the gland. The best results are obtained with mice, where without any technical difficulty perfect histological preparations of the entire gland can be obtained, showing with great clearness not only the adrenalin granules, but also the nuclei, cell outlines, blood-vessels with blood corpuscles, and the connective tissue. If such a section is immersed in a 10 % solution of hydrogen peroxide in 80 % alcohol for about 2 hours, it can be stained in the same way as a section obtained by the ordinary histological methods, and gives good preparations when stained for instance by Heidenhain's iron-hæmatoxylin method.

The normal "resting" gland.

The appearance of a normal gland of a mouse is given in Figs. 1 and 3*. It shows most of the medullary cells filled with very fine granules of adrenalin stained a dull black like coal-dust and evenly distributed throughout the cell. The nuclei show a clear karyoplasm with the network of chromatin stained grey. But there are also a large number of clearer cells which are mostly arranged in round alveoli giving the appearance of islets. These cells, which have a swollen appearance, are filled with very fine granules stained a light grey and contain in addition one or more round black "globoid bodies" or "spheroids" of varying size which are irregularly placed within the cell. They may be attached to the nuclear membrane or to the cell wall. Sometimes they can be seen lying outside the cell

* Figure 3 is actually from a mouse which had successfully resisted exposure to cold, as will be seen below (see p. 7). The appearances are so similar to those of a normal "resting" gland that the figure is referred to here as illustrating the latter condition.

altogether in the narrow intercellular spaces. These globoid bodies have a superficial resemblance, especially in size, to the lipoid globules seen in the cortex. But on closer inspection a distinct difference in the quality of the black stain can be noted, and as will be shown presently, it can be demonstrated conclusively that these spheroids are not lipoid or fat globules. These islets are not a tissue *sui generis* but represent resting medullary cells in a special stage of functional activity, probably that of a cell which has secreted its content of adrenalin and is about to refill itself again. This is to be inferred from the fact that the islets are more numerous in a gland which has been active, and that sometimes these islets are clear, while at other times they are filled with very fine granules which give them a greyish appearance but still contain the black spheroids. In the medulla of the normal mouse no, or very few, free adrenalin granules are seen in the large central vein or its tributaries. It may be added here that the animals were always kept in a warm room, handled carefully, so as not to frighten them, and killed quickly either by breaking the neck or by coal-gas. The islets of clear cells occur also in the suprarenal gland of the rat, the cat and the dog. In the glands of guinea-pigs and rabbits, of which only a small number have been examined so far, these islets of clear cells have not been seen. Nevertheless, there is also in these two species a difference in the appearance of the various medullary cells. Some stain a deep-black, while other cells have a lighter greyish appearance. It is interesting to note that of all animals examined so far, the mouse's suprarenal has the largest medulla in proportion to the size of the cortex.

The cortex of the gland is in the mouse relatively narrow. It shows an outer zone, the zona glomerulosa, about 2 or 3 layers of cells thick which is free from lipoid. Then follows the zona fasciculata which occupies the greater part of the cortex and which in its outer two-thirds shows cells filled with lipoid globules stained with a black having a glazed quality. Between this zone and the medulla there is a band of tissue which in the resting gland is very narrow. It is composed of very small round cells and of a network of capillaries. In the mouse this zone, the zona reticularis, does not normally contain any lipoid globules. If the removal of the adipose tissue surrounding the gland has been incomplete, small pieces of this adipose tissue are seen outside the gland as in figure 1. The "fat" of this adipose tissue is, as we have shown in a previous paper, rich in cholesterin and its derivatives, and resembles in its chemical composition and in the arrangement of its

"fat" globules, the "fat" of the cortex. Many of the cells of this adipose tissue, instead of being distended by one large round globule of fat, are filled with a large number of small globules.

It has been stated above that the osmic acid stained adrenalin granules and the black globoid bodies can be distinguished from the lipoids and fats by the quality of the black. The distinction can be made unequivocal by treating the section with turpentine. Turpentine dissolves fats and lipoids even after they have been stained with osmic acid. If therefore the sections are placed in turpentine for 15–30 minutes, the blackened lipoids are dissolved so that the cells now show clear vacuoles in their places. The black adrenalin granules and the spheroids in the clear islets remain almost unaltered, and only fade gradually if the section is kept in turpentine for many hours. Canada balsam has a similar but slower action, so that in sections which have been mounted in Canada balsam for some time, the lipoids have disappeared and are represented by vacuoles. Figs. 1 & 2 are drawn from a preparation which had thus been mounted in balsam without treatment with turpentine and which had been kept for 18 months. The osmic acid stained "fat" in the adipose tissue outside the cortex is a little more resistant to the action of turpentine, and is hardly affected at all by balsam in the mounted specimens, probably on account of the larger size of the globules.

Response of the gland to powerful stimulation : Lethal doses of tetrahydronaphthylamine, severe exposure to cold.

A profound change comes over the medullary cells when the suprarenal gland responds to a powerful stimulus by a massive secretion of adrenalin. This is best studied by examining mice at varying intervals after an injection of a large dose of tetrahydronaphthylamine. A gland examined half an hour after an injection of a large dose is represented in Fig. 4. The dose actually injected in this case (2·5 mg.) is sometimes tolerated by a mouse of 20 g., but frequently kills within one hour, usually with hæmorrhages into the lung. In the present case the mouse died 40 minutes after the injection with pulmonary hæmorrhage. The figure shows the central vein filled with black adrenalin granules. Many of the medullary cells have lost their cell outlines, and their cytoplasm gives the appearance of having become laked. In such cells the adrenalin granules are no longer visible, the cytoplasm being stained an even dark brown. Their nuclei have shrunk, the clear karyo-

plasm has disappeared, and the nuclei now appear as small, dark brown, elliptical bodies, so that they simulate sometimes the appearance of red blood corpuscles. They might indeed be mistaken for these but for the fact that all the intermediate stages between these forms and the large nucleus with clear turgid karyoplasm can be seen. The cells exhibiting this laked appearance are distributed irregularly throughout the gland. Other groups of cells show vacuolation with partial loss of cell outlines, so that only the nucleus with a portion of the granular cytoplasm remains. Sometimes the vacuoles of a number of adjacent cells become confluent, so that a group of medullary cells then gives the appearance of an alveolus of a gland pouring its secretion into a central lumen.

The two conditions of the medulla which have been described represent the two extremes of rest and activity. The term "rest" is used here in a relative sense, and its use is not meant to exclude a slight continuous secretion of adrenalin. The changes which the medullary cells undergo when the gland is stimulated to excessive activity are so unusual and so various that it is not yet possible to understand fully their significance. In particular the condition described above as "laking" of the cell contents and the accompanying shrivelling up of the nuclei is difficult to interpret. Is it a change from which the cell can recover, or does it signify a condition akin to cytolysis? Whatever the correct answer to this question may be, it is clear from the observations made so far, that this alteration represents an extreme condition of the medullary cells, and indicates that these cells have lost the capacity to act as adrenalin-secreting elements, at any rate temporarily. It occurs when the gland is faced with demands which are beyond the normal limits of its functional capacity. It thus sometimes occurs in the gland of a mouse subjected to an exposure to cold so severe that after a few hours the animal is unable to maintain its temperature. Fig. 5 is taken from the adrenal of a mouse which had been wetted and kept at an air temperature of about 5° C. in a glass jar, the bottom of which was just covered with water. After one hour when the animal was in a state of collapse and its temperature had fallen below 32° C., it was transferred to a warm room where it soon revived, and was kept for two hours. It was again exposed to cold for one hour and then killed. In the figure a part of the medulla around the central vein has been selected showing a large number of these laked cells, together with other vacuolated cells which have been emptied of most of their adrenalin granules. Similarly the injection of a large dose of tetra-

hydronaphthylamine, which also, as stated above, produces this condition of laking in many cells of the medulla, must be looked upon as an excessive stimulus. For it leads to a secretion of adrenalin so massive that the lungs become deeply congested, and animals often die with hæmorrhages into the lung just as if a lethal dose of adrenalin had been injected. These laked cells are also abundant in the medulla of mice which have been kept at a temperature of about 40–45° C. The animals appear to be unable to adapt their heat regulating mechanism to such a temperature, and succumb after 24 to 36 hours. Mice which have been fed with a large dose of thyroid gland also show sometimes these laked cells in the suprarenal medulla.

Response of the gland to a slight stimulation : Exposure to cold of moderate degree.

The normal process of secretion of the medullary cells in response to a stimulus of moderate strength is represented by the formation within the cells of vacuoles containing adrenalin granules described above, and the passage from those into the central vein.

This change does not affect all the medullary cells equally, so that a great part of the medulla does not differ materially from that of a "resting" gland. In fact, since a slight continuous secretion is taking place in the so-called "resting" gland and adrenalin granules may sometimes be seen in the central vein of such a gland, the difference between a "resting" gland and one responding to a slight stimulus cannot be clearly defined.

Fig. 3 represents a high-power view of the gland from a mouse which had been exposed for nine consecutive nights to dry cold, being kept in a warm room during the day time. At the end of that period it was perfectly well and was killed. The figure represents the medulla near its junction with the cortex of which the innermost layer is visible. The appearance of the medullary cells is indistinguishable from that of a normal resting gland, and has been selected to show one of the islets with the black globoid bodies described above It may be noted here that the cells of the zona reticularis show a distinct change : they are larger and more swollen than the cells of a normal animal, and one of the cells contains a number of small black globules which are not fat or lipoids. This will be referred to again below.

Self-regulation of the "load" of adrenalin.

A peculiar and important feature of the activity of the suprarenal gland is the flexibility with which the gland responds to a stimulus not only by the secretion of its hormone but also by a corresponding new formation. The result is that with a slight or even moderate stimulus the gland remains always loaded although it is continuously secreting. This is demonstrated very clearly by applying such a stimulus as cold in a moderate degree. Individual mice differ greatly in their resistance to cold. Under identical external conditions, involving the moderate application of the stimulus of cold, some animals remain well, while in others the temperature of the animal falls, and if exposure to cold is continued the animal dies. In the latter animals the medulla shows marked depletion of the store of adrenalin, while the medullary cells of animals which have remained in good health under the same conditions are fully charged with the fine black granules of adrenalin. Since in both groups of animals there is an increased secretion of adrenalin, the difference in the condition of the medullary cells must be ascribed to the fact that in the animals which did not resist exposure to cold, the rate of adrenalin formation did not keep pace with the rate of adrenalin secretion. The death of these animals is clearly related to this failure on the part of the suprarenal gland. In the animals which did not succumb, the increased secretory activity of the medullary cells must have increased the processes of adrenalin formation to such an extent that the adrenalin store in the medulla was maintained. These results recall the observations of Elliott and of Stewart and Rogoff on the effects of splanchnic stimulation. These authors showed that splanchnic stimulation did not diminish the load of adrenalin in the medulla, although it produced a distinct secretion of adrenalin into the blood stream. In the striking experiments of Stewart and Rogoff the amount of adrenalin given off by the gland as the result of splanchnic stimulation during four hours was not less in amount than the load of a single land. Nevertheless, no deficiency in the store of the stimulated gland was found.

Depletion of the load of adrenalin.

If the stimulus is strong and prolonged so that the maximum secretion of adrenalin with the intense cellular changes described above is produced, the new formation of adrenalin may not be able to keep pace with the rate at which it is poured out into the blood stream. Then a

partial depletion of the gland occurs, from which it may recover when the stimulus is withdrawn. This course of events has been studied experimentally by injecting a large dose (2·5 mg.) of tetrahydronaphthylamine into a number of mice and examining their glands at half-hourly intervals. In such an experiment the load diminished during the first two or three hours, while an active secretion of adrenalin continued. Then the medulla gradually became again replenished, so that after six hours it had almost regained its normal appearance.

A more extensive depletion of the gland occurs, as stated above, when a mouse is exposed to cold and is unable to maintain its normal body temperature. If the mouse with the subnormal temperature and depleted medulla is brought into a warm room and the stimulus is thus withdrawn, it may recover and then the medulla becomes again loaded with adrenalin. But if exposure to cold continues the mouse eventually dies. A low power view of the suprarenal of such an animal, which was killed in a state of collapse after having been exposed to wet and cold for four hours, is shown in Fig. 2. The medulla is almost free from adrenalin and contrasts sharply with the fully loaded medulla of a mouse also exposed to cold, which was able to maintain its body temperature (Fig. 3). A high power view of the medulla from the gland of a different mouse exposed to severe cold is given in Fig. 5, which has already been discussed with reference to the presence of the "laked" cells. This figure also shows a number of normal cells with a clear turgid nucleus. These cells differ from those of a normal resting gland in having lost almost completely their load of adrenalin granules and many of them are deeply vacuolated.

Changes in the cortex accompanying activity of the medulla.

The activity of the medullary cells is accompanied by changes in the cortex, particularly in the zona reticularis. This zone becomes deeply congested and the congestion extends to the medulla. The cells of the zona reticularis increase in size, so that the zone as a whole expands and instead of a narrow thread now appears as a broad band (see Fig. 2). What is even more significant is the appearance in the cells of this zone of small globules staining black with osmic vapour which are not fats or lipoids (see Fig. 3). These small globules must be distinguished from large globules of fat or lipoid material which are sometimes seen in this zone in a gland which has

been very active. The occurrence of these large globules of fat or lipoid is, however, infrequent and the application of turpentine makes the difference clear.

Whether these small globules indicate the presence of adrenalin itself in this zone of the cortex or whether they represent a precursor of adrenalin cannot be decided merely on the ground of this histo-chemical reaction. But their appearance in this zone when the medulla is stimulated to activity and other changes which take place in the cortex and particularly in this zone, such as the swelling of the cells in the zona reticularis and the congestion, indicate that the cortex and medulla act together so far as the formation of adrenalin is concerned and are not, as has been suggested, two organs functionally quite independent of each other. It is interesting to note that in such a gland which has been depleted by a severe exposure to cold, the lipoids of the cortex are not diminished to any great extent, while at the same time the fat may have disappeared almost completely from the adipose connective tissue depots. This independence of the cortical lipoid agrees with similar observations made by Elliott on human material.

Significance of the "load" of adrenalin.

It is clear from what has been stated so far, that the so-called load of adrenalin in the gland represents merely the balance of formation and excretion, and gives only very incomplete and perhaps even misleading information concerning the functional activity of the gland. That is determined by the rate at which adrenalin is being secreted and being newly formed in the gland. It may be pointed out in passing that if the load is determined by Folin's chemical test, a fallacy is introduced by the varying congestion of the gland in different conditions of functional activity. As Folin himself has pointed out, the colour reaction on which the estimation depends is not specific for adrenalin, but is given also by blood. Even if this fallacy be disregarded the load of an actively secreting gland may approach that of a normal resting gland, or it may be partly depleted while the gland still continues to secrete. The first condition may be described as an "active loaded gland," the second as an "active depleted gland." The extent to which a gland is depleted depends naturally on the strength of the stimulus and the length of time during which it is applied. A prolonged, strong stimulation will lead to a complete depletion, so that the gland becomes "exhausted."

As an example may be given exposure to cold so severe that the animal is unable to maintain its body temperature (see Fig. 2).

It is clear that a depleted gland which is still able to form new adrenalin may be capable of maintaining the life of the animal if the demand for adrenalin is diminished, whereas a similarly depleted gland in which the process of formation of adrenalin has been inhibited may be incompatible with the maintenance of life. The value of the histo-chemical method lies in this fact that it not only affords information with regard to the load of adrenalin, but reveals also the finer cellular changes which indicate the state of functional activity of the gland.

Effect of acidosis, hæmorrhage, and anæsthesia.

There is evidence that exhaustion of the gland may occur also without an antecedent hyperactivity of the gland. It must then be due to an inhibition of the new formation of adrenalin and may therefore be described as a "paralysis" of the gland. When 0·5 c.c. of $\frac{N}{10}$ hydrochloric acid is injected intravenously into a mouse it shows at first symptoms of being seriously ill. It quickly recovers however, and when killed half an hour after the injection, when it is apparently well, the suprarenal medulla is extensively depleted. The probable explanation is that adrenalin, being a base, has been washed out by the acid with which it has formed a salt and that the new formation of adrenalin had been inhibited. Similarly a big hæmorrhage produces a rapid and extensive depletion of the gland. This depletion is so rapid that it cannot be accounted for by a massive secretion of adrenalin alone, although an active secretion is demonstrable histo-chemically. Here the loss of blood and the resultant insufficient oxygenation of the gland cells are probably instrumental in inhibiting the new formation of adrenalin. A less extensive but nevertheless distinct depletion of the gland occurs as the result of anæsthesia itself without any operative procedure, especially in chloroform anæsthesia. This effect is not accompanied by a hyperactivity of the gland. The experiments were made on mice which were anæsthetised for 25 minutes with chloroform or with ether and then killed with the anæsthetic. The animals were kept warm with a hot water bottle and cotton-wool. Nevertheless, their rectal temperature fell.

Effect of bacterial infections : gas gangrene, streptococcal abscess, streptococcal cellulitis.

Another condition which produces an exhaustion of the suprarenal medulla without an antecedent hyperactivity is infection with the bacteria of gas gangrene or with their toxins, and in such a gland the lipoids disappear from the cortex. The practical importance of this paralysis of the gland in gas gangrene has been discussed in greater detail in the following paper in the chapter "On the lethal mechanism of gas gangrene." But it may be pointed out here that the state of collapse, with subnormal temperature, which is a clinical feature of the terminal stage of gas gangrene, may thus be accounted for as the result of adrenal insufficiency. Figure 6 represents the gland of a mouse which had received subcutaneously 0·5 c.c. of Welch toxin (min. lethal dose 0·3 c.c.) and died 20 hours afterwards.

Gas gangrene is a bacterial infection characterised by the absence of prolonged pyrexia and terminating with hypothermia. It was of interest therefore to study the suprarenal in a bacterial infection which is associated with a hyperthermia. The conception of the thyroid-adrenal apparatus outlined at the outset of this paper would lead one to expect that such bacterial infections would stimulate the secretion of adrenalin and differ in this respect from the paralytic effect of the bacteria of gas gangrene. This point has been tested with a streptococcal infection. A local abscess was produced in mice by the injection of a suspension of streptococci in saline and a spreading streptococcal cellulitis by the injection of a suspension of streptococci in calcium nitrate. The strain used was not very virulent, and although the cellulitis made the animals obviously ill, none died within four days. In all these animals the adrenals were found to be secreting adrenalin actively, the cortical lipoid was not diminished, and the picture presented was generally similar to that obtained after a small dose of tetrahydronaphthylamine. In larger animals (rats, rabbits) such an infection produced a slight pyrexia lasting for three or four days.

Observations were also made with a more virulent strain of streptococci. When a cellulitis was induced with this strain three out of four animals died within four days in a condition of collapse. When a circumscribed abscess only was produced the animals did not appear to be ill. The experiment is identical with the one discussed in detail in another paper on p. 67 of this Report. In all these animals the suprarenals were

much more severely affected than with the less virulent streptococcal infection. In the mice with a circumscribed abscess the medulla was partially depleted but the cells were still actively secreting. The cortical lipoid was not obviously diminished. In some of the animals with cellulitis the glands were completely exhausted and then the cortical lipoid had disappeared just as in gas gangrene. Elliott has shown in human material that the cortical lipoid disappears in many acute septic infections. Our observations, which confirm those of Elliott, show that this occurs only in the terminal stage. If intermediate stages are examined the lipoid was found to have disappeared first from the outer layers of the zona fasciculata, while it appeared in the inner layers of this zone and even in the zona reticularis. There is thus a transportation of the lipoids from the periphery towards the medulla, but not into the medulla itself.

The exhaustion of the gland in the bacterial infections mentioned above differs from that occurring in other conditions, such as exposure to cold, for instance. In the former adrenalin disappears from the medulla together with the cortical lipoid. In exhaustion due to cold the lipoids remain in the cortex. It is true, therefore, that "the load of lipoid is not directly related to that of adrenalin," as Elliott states it. But it is also true that the conditions in which the cortical lipoid does disappear are always those in which there is also a depletion of adrenalin from the medulla. Facts have been given above which indicate that one of the functions of the cortex is to assist the medulla in the formation of adrenalin. Presumably a condition in which complete depletion of the medulla is associated with a cortex depleted of lipoids represents a more advanced stage of exhaustion from which recovery is not possible.

Changes in human glands in cases of acute pyrexia.

A number of human thyroid and suprarenal glands from a variety of cases associated with a pronounced pyrexia have been examined. For this material I am indebted to the courtesy of Dr. Lawrence of University College Hospital and to Dr. Spilsbury of St. Mary's Hospital. This material was examined before the osmic vapour method had been devised, which in any case could not usefully be applied to human material obtained 24 hours after death. Only formalin-fixed material has therefore been examined. Such material fails to give any clear

detailed information on the activity of the medullary cells and shows only gross changes in the gland. Even with material from experimental animals obtained immediately after death and fixed by the ordinary fixatives (Formol-saline, Zenker's fixative) no histological details can be seen in the medulla, if the gland has been actively secreting at the time of death. As an example two figures of the medulla of glands from rabbits fixed in Zenker are given. Both were taken from rabbits immediately after death. Fig. 7 represents a normal resting gland. Such a gland gives good histological details. Fig. 8 is from an animal which had received an injection of 0·08 g. tetrahydronaphthylamine and died two hours later with a temperature of 44° C. The medulla is congested. The cells which are deeply vacuolated, and have lost their outlines are widely separated from each other and appear simply as irregular shreds of protoplasm around a nucleus. Most human adrenals obtained from the post-mortem room have this appearance. It gives the impression of an exhausted gland and that was the interpretation we put upon it before the osmic vapour method had been devised. But comparison with this latter method shows that the picture presented by Fig. 8 is that of an active gland.

In post-mortem material the most significant indication of an actively secreting gland is a congestion of the gland affecting especially the medulla and the zona reticularis. Two cases are of special interest: one from a child, the other from an old man. Both developed suddenly a hyperpyrexia and died. At the post-mortem examination no obvious cause was found to account for the sudden rise in temperature. The suprarenals which had been fixed entire, showed on section an intense congestion and hæmorrhages into the medulla.

In this connection reference may be made to recent observations on human post-mortem material in two conditions which are characterised by a severe and acute pyrexia, namely, malaria and influenza. In both these conditions a congestion of the gland has been observed which was most intense at the junction of cortex and medulla, and which, sometimes, produced hæmorrhages in the gland (Dudgeon and Clark, Paisseau and Lemaire in malaria; Wolbach, Winter in influenza). Unfortunately, the thyroid gland was not examined by any of these observers. As the following observations show, it is probable that in both these conditions distinct changes take place also in the thyroid gland.

Some changes in the thyroid gland associated with activity of the suprarenal gland.

In man owing to the difficulty of obtaining suitable material for the study of the suprarenals the activity of the thyroid-adrenal apparatus is more usefully studied by examination of the thyroid gland. Since this paper deals only with the suprarenal a detailed description of the changes in the thyroid gland which accompany the activity of the suprarenal must be reserved for future publication. Reference has already been made to the intense congestion of the thyroid and the partial disappearance of the colloid which occurs in the experimental hyperpyrexia produced by the injection of tetrahydronaphthylamine, and it may be added that there is frequently produced an extensive desquamation of the cells lining the thyroid alveoli. As an example of the similarity between the effect on the thyroid-adrenal apparatus of the experimental fever produced by tetrahydronaphthylamine and the severe fever resulting from a bacterial infection the following two figures are given. Fig. 9 gives the appearance of the thyroid of a rat after an injection of 0·02 g. tetrahydronaphthylamine. The animal died two hours later with pulmonary hæmorrhages. Fig. 10 represents a human thyroid from a case of meningitis with prolonged and severe pyrexia. Macroscopically this gland showed no obvious abnormality. The similarity in the microscopic appearance of these two glands is obvious. In this connection reference may be made to the interesting results obtained by Dr. J. A. Murray as the result of a systematic examination of the thyroid glands of warm and cold-blooded animals which had died in the Zoological Gardens as the result of severe infections. Dr. Murray reports as follows :—" In warm-blooded animals dying under these conditions extreme congestion of the whole gland is practically constant. Nothing of the kind has been encountered in the reptiles examined, although a large proportion presented severe septicæmic conditions after death. This result is unfavourable to the view that the thyroid plays the part of a neutraliser of toxic substances in the body. It is in much better harmony with the view that the changes in the thyroid in these conditions are the expression of its participation in the heat-regulating mechanism of the body. In poikilothermic animals one would expect these changes to be absent."

The suprarenal gland in post-operative shock.

The condition of the suprarenal gland in post operative shock was investigated in mice which in the course of an abdominal operation had developed this condition as the result of excessive handling of the intestine. Incautious pulling of the mesenteric attachment is particularly apt to induce shock. In most of these animals there was no hæmorrhage at all. The splanchnic vessels were engorged so that the intestine appeared suffused with dark venous blood ; the temperature became subnormal and did not rise to the normal even when the animal was kept very warm, breathing was slow and laboured, and death ensued as a rule within a few hours. A mouse in this condition of post-operative shock resembles a mouse dying from cold, hæmorrhage, or gas gangrene. One would expect therefore to find the suprarenal to be exhausted, as it is in the conditions just mentioned. But the reverse is the case. The cells, it is true, are extensively vacuolated, but they are loaded with adrenalin granules and are actively secreting these granules into the central vein at the very moment when the animal dies with low blood-pressure and sub-normal temperature. This fact, surprising as it is, is supported by the observations of Bedford and Jackson and by the later work of Stewart and Rogoff by means of the biological test for adrenalin. The former authors claim to have demonstrated a greatly increased adrenalin content of the blood in this condition. Stewart and Rogoff, who used a more accurate method for the biological assay of adrenalin, deny that the adrenalin content of the blood is increased in shock and show that it is the same as that of a normal animal. But all observations agree in demonstrating that the condition of shock is not associated with a failure of the suprarenal gland to secrete adrenalin.

General Discussion.

That such diverse conditions as exposure to cold and infection by certain bacteria, such as for instance streptococci, produce an increased functional activity of the suprarenal gland is readily understood on the basis of the conception of the thyroid-adrenal apparatus as an important factor in the heat regulation of the animal. For in both these conditions there is an increased activity of the heat regulating mechanism. Although in this paper no detailed account is given of the changes in the thyroid gland it may be stated that in both these conditions there is an increased activity of the thyroid gland. The most obvious and

constant indications of this are the intense congestion of the gland and the changes in the colloid, which loses its affinity for basic stains and partly disappears.

It is clear, therefore, that climatic conditions exercise a strong influence on the thyroid gland and through it on the suprarenal gland. The significance of this conclusion is apparent when one considers the widespread influence which is exercised by these glands on metabolic processes, on the sympathetic nervous system, and in man at any rate, on the mental activities.

Bacterial infections as such do not have a uniform action on the adrenal gland : for while a streptococcal infection producing an abscess may stimulate the activity of the gland, gas gangrene ending in the death of the animal paralyses it. This difference is reflected in the temperature reaction. It does not necessarily follow that the difference between the exhausted paralysed suprarenal in gas gangrene and the actively secreting loaded gland in a streptococcal infection is due to an intrinsic difference between the actions of the respective toxins of these two groups of bacteria. If that were so, it would be possible to distinguish between bacterial toxins which stimulate the suprarenal, and those which paralyse it. Such an idea is very attractive, but it is also possible that all bacterial toxins act in a similar manner on the suprarenal gland and that the difference between the actively secreting gland in one infection and the paralysed gland in another is due to the varying degree of intoxication. Thus gas gangrene is characterised by the rapid and massive nature of the toxæmia produced by it, which may kill the animal in 24 hours. A streptococcal abscess, on the other hand, produces only a slight toxæmia which does not even make the animal obviously ill. And a streptococcal cellulitis which produces obvious constitutional symptoms, does not necessarily kill the animal. When, with a more virulent strain, the animal dies, the gland is paralysed as in gas gangrene. One may argue that the more virulent strain has produced toxin more rapidly, or one may urge that it has produced a different toxin, or an additional toxin, such as a hæmolysin. In gas gangrene different features of the infection, such as the production of an acidosis and of a hæmolysin, contribute to the paralysis of the gland, as is shown in the following paper in the chapter "On the lethal mechanism of gas gangrene." An investigation of the pharmacological action of the different toxins would throw further light on this question. It would in particular be important both from the practical and from the theoretical point of view to know whether

bacterial toxins which lead to a paralysis of the gland, such as those of gas gangrene, produce their effect entirely by an action on the suprarenal medulla, or whether they also paralyse the sympathetic nerve endings or nerve cells.

There is yet another possibility which has to be borne in mind in correlating the condition of the suprarenal gland with the general condition of the animal as a whole. From the close functional relationship between the thyroid and suprarenal glands which we have demonstrated, it follows that the functional capacity of the latter gland is determined also by the condition of the thyroid gland and should not be viewed only by itself. A similar degree of exhaustion of the suprarenals of two animals may nevertheless have a very different significance with reference to the maintenance of life, if it is associated in the one case with an exhausted thyroid, and in the other with a thyroid gland which is still capable of functional activity. Unfortunately we do not possess a histo-chemical test for the functional activity of the thyroid so that it is at present difficult to interpret with certainty changes which would indicate an exhaustion of the gland.

At present one must be satisfied, therefore, to draw the general conclusion with reference to the bacterial infections dealt with in this paper, that in these infections, and probably in many others, the action of the bacterial toxins falls upon the thyroid-adrenal apparatus. This action explains many clinical features of these infections, in particular the temperature reaction : pyrexia indicates a hyperadrenalism, while a subnormal temperature signifies exhaustion of the gland. On recovery from a bacterial infection which has been accompanied by a severe pyrexia such as occurs for instance in influenza there is a state of depression and there may even be a subnormal temperature. This, in the light of the conception outlined in this paper, is readily understood as being the result of an exhaustion of the thyroid-adrenal apparatus due to over-stimulation. If this is correct it should be possible to counteract this state of depression by the administration of thyroid gland.

In dealing with gas gangrene reference has been made to the fact that the paralysis of the gland in that condition is due to the simultaneous action of a number of different factors, namely a toxæmia, an acidosis and the production of a hæmolysin. It is a matter of general importance to realise that a number of very different conditions when acting together on an animal will reinforce each other by their joint action on the suprarenal gland,

To any one of these when acting alone, even for a prolonged period, the suprarenal gland may be able to respond with an increased activity, so that even with a gland temporarily depleted the animal may resist the condition without manifesting serious symptoms. But a combination of two or more such conditions operating together, perhaps only for a short time, may overtax the functional capacity of the gland and produce a complete exhaustion or even paralysis of the gland, and consequently a state of the animal from which it may only be able to recover with great difficulty. In this connection it is of special interest to note the effect of acidosis on the gland, since recent work on acidosis has tended to belittle the importance of this condition. The fulminating effect which may thus be produced by a combination of various conditions all of which operate on the suprarenal gland may suitably be described as an "avalanche-effect."

Clinical experience as summarised by Mr. Cuthbert Wallace in the Report on Shock published by the Medical Research Committee has led to an almost identical conclusion. Mr. Wallace writes as follows (p. 7): "Shock is usually the result of several factors such as hæmorrhage, toxins, cold and anæsthetics. The effect produced depends on the intensity of each, on the number that are acting at any one time, and on the duration of the period during which they are operative. Any one of these factors encountered in a moderate degree and for a limited period can be dealt with by the body.... If two occur together then the effect produced is more than the sum of the two acting separately and the body has difficulty in righting itself. If to these two factors be added a third recovery may still be possible. On the other hand a fatal result may ensue if the body is further taxed by the administration of an anæsthetic which to a normal individual would be comparatively harmless." The observations recorded in this paper enable us to understand the etiological connection between these various conditions. They also show—and in this respect our conclusions differ from the statement quoted above—that there is a condition of shock which is not due to any of the factors mentioned, acting severally or jointly. Not only is this condition of post-operative shock different etiologically, but in view of the state exhibited by the suprarenal gland it must be looked upon as a condition *sui generis*, although it exhibits the same complex of symptoms. In post-operative shock the suprarenal gland is loaded with adrenalin and actively secreting it at the very moment when the animal dies with a low blood pressure and a subnormal temperature. Evidently then in this condition of shock the symptoms are due to the fact that the

animal is incapable of responding normally to the action of adrenalin. In hæmorrhage, gas gangrene and exposure to cold these symptoms develop primarily as the result of exhaustion of the gland. This difference is obviously of importance both from the point of view of the etiology and of the treatment of the condition. It is advisable therefore to distinguish true shock from these other conditions associated with exhaustion of the gland which as a clinical entity might suitably be grouped together under the term collapse. The condition of the suprarenal gland in post-operative shock affords an explanation why adrenalin as a therapeutic measure has been useless in shock. But in collapse, as defined above, the administration of adrenalin may prove to be a more useful measure, unless the condition has lasted so long as to lead to a paralysis of the nervous centres or nerve endings as the result of the prolonged low blood pressure.

These observations also emphasize the importance of the application of warmth in the treatment of wounded men. The conditions of modern war put a more severe strain on the suprarenal gland than probably any other organ in the body. The strain of which this gland can be relieved most easily when a wounded man comes under treatment is that concerned with the heat regulation of the body.

Summary.

The ease with which the action of massive doses of adrenalin on the blood pressure can be demonstrated has dominated unduly the conception of the function of the suprarenal gland in health and in disease. The observations recorded in this paper show that the gland, and in particular the medulla, is profoundly affected in a number of conditions associated with disturbance of the heat regulation of the body, namely exposure to cold, fever and bacterial infections of varying severity. Changes in the functional activity of the medulla have been observed also in experimental hyperthyroidism, experimental acidosis, severe hæmorrhage, anæsthesia and post-operative shock.

Changes in the medulla of the gland produced by some of these conditions are accompanied by changes in the cortex and in the thyroid gland which are briefly described.

The observations furnish further evidence in support of the conception that the thyroid and adrenal glands form an apparatus for the heat regulation of the body.

The suprarenal gland is therefore an organ, the functional capacity of which may be called into action by a great variety of different

conditions, of which the one connected with the heat regulation of the body is almost always operative. It is enabled to meet these requirements by virtue of the fact that the secretion of adrenalin by the gland is in itself a stimulus to the new formation of adrenalin, so that the functional activity of the gland does not necessarily involve a depletion of its load of adrenalin. But the new formation of adrenalin may be inhibited, or excessive demands may be made upon the gland, as occurs for instance when several of the conditions mentioned above operate at the same time. Then the gland is exhausted and collapse ensues. This condition of collapse is different from that of post-operative shock, which although exhibiting the same symptoms is associated with a loaded and actively secreting gland.

It is pointed out how these conceptions bear upon the rational treatment of the various conditions.

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ON THE MECHANISM OF BACTERIAL INFECTIONS, WITH SPECIAL REFERENCE TO GAS GANGRENE.

By W. E. BULLOCK and W. CRAMER.

(Plates VI.–X.)

I. On the Lethal Mechanism of Gas Gangrene and on the importance of accessory factors in relation to it.

The condition of gas gangrene may be briefly summarised as a severe local lesion accompanied by a toxæmia and followed frequently by a septicæmia. But none of these three factors is in itself sufficient to account for the fulminating rapidity with which the condition may lead to a fatal issue, as the following considerations will show.

In what follows it is assumed on the basis of the work of McIntosh (5) and Weinberg (7) and others that the organisms chiefly responsible for gas gangrene are B. Welchii, B. œdematiens, and *vibrion septique*. Most of our observations were made with B. Welchii on mice, which are a species susceptible to that organism. It is easy to show that the site of the lesion is not a decisive though it is an important factor. Thus with a strain of B. Welchii of low virulence, a dose which will kill an animal within 24 hours when injected intramuscularly, will not kill or even make the animal seriously ill when injected subcutaneously. For instance: 0·25 c.c. of a 20-hour culture of B. Welchii given intramuscularly killed a mouse of 15 grammes, while the same dose of the same culture did not kill when given subcutaneously. By increasing the dose or by using a virulent strain it is however possible to kill an animal by subcutaneous injection of B. Welchii. In such an animal there is at the site of injection an extensive hæmorrhagic œdema with a varying degree of necrosis in the subcutaneous tissue. The macroscopic changes in the tissues remote from the site of injection are identical with those following upon injection into the leg muscles and are as follows:—the kidneys are deeply congested, the bladder frequently

contains a dark brownish-red fluid, probably altered blood, the liver is dark and small with a gall-bladder distended with dark bile, the spleen is small, the adrenals are deeply congested, the duodenum is dilated and has an intense yellow-pink colour, the thyroid is small, the lungs are congested.

That the mere circulation of the organisms in the blood is not, in itself, an important factor in bringing about a lethal issue, can be readily demonstrated. For if a dense emulsion (in saline) of bacteria from a surface culture is introduced intravenously into mice the animals remain unaffected and in good health, even although a blood-culture may demonstrate the presence of bacteria in the heart blood for at least two days after the injection.

The existence of the second factor mentioned above, namely the toxæmia, is now established beyond doubt. For it has been possible to obtain from a broth culture of B. Welchii a toxin free from bacteria which in suitable doses will kill a mouse with lesions similar to those produced by injection of the living organisms. Moreover, the animals can be protected against the lethal action of both the toxin and the living bacteria by the injection of an antitoxin.

So far the facts appear to support the view that the production of toxins by B. Welchii and *vibrion septique* is an adequate explanation of the condition of gas gangrene. It is, however, difficult to account on the basis of this conception for a number of facts of which some are of considerable practical importance in the treatment of the condition. An objection which is academic in nature is that the toxins prepared hitherto from various strains of these two organisms are relatively weak. In the case of Welch toxin for instance, prepared from different strains, the minimal lethal dose has never fallen below about 0·1 c.c. for a 15 gramme mouse. In this respect this toxin offers a striking contrast to the toxins of tetanus and diphtheria, which require very minute doses in the case of a susceptible species. Another difficulty lies in the fact that the presence of non-pathogenic bacteria in gas gangrene, such for instance as B. sporogenes which, when injected alone, produces only slight local lesions, greatly adds to the severity of the condition. A consideration of these facts suggests that there must be additional factors which determine the lethal issue in gas gangrene. A consideration of the lethal mechanism of gas gangrene affords an explanation of some of these perplexing and apparently paradoxical features of the condition.

It has been found that in animals dying of gas gangrene as the result

of inoculation of a broth-culture of either B. Welchii or *vibrion septique* there is a lasting exhaustion of the suprarenal glands, as demonstrated very clearly by the osmic vapour method (see preceding paper, Pl. III. fig. 6). The chief changes are congestion of the cortex and great diminution of the cortical lipoid, while the medulla shows an almost complete disappearance of adrenalin. Similar changes have been observed in the suprarenal glands of men dying from gas gangrene (4). It is the change in the medulla which is of special significance since a diminution of the cortical lipoid occurs, as Elliot has shown, in a number of septic conditions of diverse origin. The term "lasting exhaustion of the suprarenal gland" indicates not only the disappearance of adrenalin from the suprarenal medulla, but also the inability to reform new adrenalin. The effect may be suitably described as an "inhibition" or a "paralysis" of the gland. It resembles the action of diphtheria toxin on the adrenal gland of the rabbit and guinea-pig, but differs from it in the remarkable insusceptibility of the mouse to the action of diphtheria toxin. This inhibitory effect on the gland is diametrically opposed to a stimulation of the gland such as may be produced by the injection of tetrahydronaphthylamine (referred to as "T.H.N." for the sake of brevity in the following pages), or in a lesser degree by typhoid vaccine. When the suprarenal gland is stimulated by T.H.N. there is a massive excretion of adrenalin into the blood, demonstrable histochemically. The secretion of adrenalin by the gland acts as a stimulus for its new formation. Although the amount of adrenalin present at a given moment in the medulla of the gland—the "load" of adrenalin—may be greatly diminished, adrenalin is constantly being reformed and poured into the blood-stream until the stimulation of the gland ceases and the medulla is refilled again with adrenalin to its normal capacity.

The difference between stimulation and paralysis of the gland is reflected in the behaviour of the heat regulation of the animal in these two conditions. In the preceding paper it has been shown how the suprarenal gland is concerned in the heat regulation of the organism. When the secretory activity of the gland is increased the resultant stimulation of the sympathetic nerve-endings leads to a constriction of the blood-vessels and therefore to a diminution of the heat loss. At the same time there is an increased heat production owing to the mobilisation and subsequent oxidation of the liver glycogen. The stimulation of the gland by T.H.N. produces thus a very intense hyperpyrexia. Conversely the toxin of B. Welchii produces a profound hypothermia.

In rats and mice the temperature may fall below 95° F., so that the change can be readily felt when the animal is handled. Injection of a broth-culture of the living organism produces at first a slight pyrexia which is followed by a hypothermia if the animal succumbs to the infection. Whether this is due to the absence of adrenalin, or whether there is a paralysis of the sympathetic nerve-endings is a matter for further investigation.

The effect of the toxin of gas gangrene is therefore one which tends to lead to an exhaustion and paralysis of the suprarenal glands. If this action is accepted as an important element in the lethal mechanism of gas gangrene, it is evident that any factors which put an extra strain on the adrenal will add to the severity of the condition.

From the observations recorded in the preceding paper it is known that the following conditions affect the functional activity of the suprarenal glands :—

1. Some conditions associated with disturbances of the heat-regulating mechanism of the body, namely :—
   (*a*) Exposure to cold.
   (*b*) Production of a certain type of fever such as can be produced by the injection of tetrahydronaphthylamine or in a less marked degree by the toxins of certain bacteria (streptococci, b. typhosus).
2. Hæmorrhage.
3. Experimental hyperthyroidism produced by feeding with large doses of thyroid gland.
4. Experimental Acidosis produced by the injection of acids.

All these conditions produce a temporary depletion of the load of adrenalin, which varies in the different conditions not only in degree but also in the manner in which it is brought about. For the purpose of the present investigation it is not necessary to discuss these differences, and it is sufficient to state that in all these conditions obvious changes can be demonstrated histochemically in the gland by the osmic vapour method.

The coexistence in gas gangrene of any of these conditions may therefore be expected to add to the severity of the infection. The experimental test shows that this expectation is fulfilled. Animals (mice) which have been inoculated with sublethal doses of Welch toxin or of broth-cultures of living B. Welchii will succumb if they receive at the same time sublethal doses of T.H.N., or of a dilute acid, or if previous to the infection they have been subjected to an exposure

to cold sufficiently long and severe to reduce the temperature of the animal, but from which the animal is able to recover if placed again under normal conditions.

Some of the experiments may be given in detail as typical examples.

Effect of exposure to cold on inoculation with broth-cultures of B. Welchii.

Experiment 30/7. 7 mice were kept without food for 24 hours and exposed to cold by being kept in a cold room at about 12° C. in separate glass jars. During the last hour the bottom of the jars were kept wet with water. At the end of that period the animals had a subnormal temperature but were otherwise well and recovered rapidly when placed in dry cages in a warm room. Of the seven mice three were kept as a control batch without inoculation—"Control cold mice." The remaining four mice received a sublethal dose (0·25 c.c.) of a 20 hours' broth-culture of B. Welchii, two mice intramuscularly into the hind leg, two mice subcutaneously—"Welch + cold mice." They were then transferred to dry cages and kept in a warm room together with the "control cold mice." A third batch of four mice, which had been kept fed and in warm dry cages, received similar inoculations with a broth-culture of B. Welchii—"Control Welch mice."

		Days after inoculation.	
		1.	2.
"Welch+cold mice."			
Mouse No. 1.	0·25 c.c. B. Welchii subcutaneously.	Alive and very ill next morning, + at 5 p.m. same day.	
„ 2.	do.	+ next morning.	
„ 3.	0·25 c.c. B. Welchii intramuscularly.	+ next morning.	
„ 4.	do.	+ next morning.	
"Cold mice."			
Mouse No. 5.		Alive and well.	Alive and well.
„ 6.			
„ 7.			
"Welch control mice."			
Mouse No. 8.	0·25 c.c. B. Welchii subcutaneously.	Alive and fairly well; coat slightly ruffled.	Completely recovered.
„ 9.	do.		
„ 10.	0·25 c.c. B. Welchii intramuscularly.		
„ 11.	do.		

The post-mortem examination of mice 1–4 showed in every case the picture of typical severe gas gangrene.

Effect of exposure to cold on the action of the toxin of B. Welchii.

A similar result is obtained with injection of the toxin : sublethal doses of the toxin become lethal for mice previously exposed to cold. The toxin used in these experiments was one which just killed in doses of 0·5 c.c. for a 15 gramme mouse. A dose of 0·3 c.c. was sublethal for mice kept under normal conditions but was lethal for mice previously exposed to cold.

Effect of acidosis on the action of the toxin of B. Welchii.

Experiment 23/8. 12 mice of equal weight (14–15 grammes) were divided into three batches of 4 mice. One batch received subcutaneously in the flank sublethal doses of Welch toxin (0·3 c.c.). The second batch received subcutaneously on the back sublethal doses of a 10 per cent. solution of lactic acid (0·2 c.c.). Such a dose frequently produces a sore at the site of injection, the skin being digested by the acid within 24 hours, but unless the sore is very extensive the general health of the animals is not impaired thereby. The third batch received both sublethal doses of Welch toxin and four hours later sublethal doses of lactic acid, the former being injected in the flank, the latter on the back.

Toxin alone 0·3 *c.c.*	*Result next day.*
Mouse No. 1.	Alive and well.
„ 2.	do.
„ 3.	do.
„ 4.	Ill.
Acid 0·2 *c.c.*+ *Toxin* 0·3 *c.c.*	
Mouse No. 5.	+, sore on back.
„ 6.	+, no sore on back.
„ 7.	+, do.
„ 8.	+, do.
Acid alone 0·2 *c.c.*	
Mouse No. 9.	Alive and well, sore on back.
„ 10.	do.
„ 11.	do.
„ 12.	Alive and well, no sore on back.

Effect of injection of "T.H.N." on inoculation with broth-cultures of B. Welchii.

Experiment 25/7. 12 mice were divided into three batches of 4 mice in each. One batch received a sublethal dose 0·25 c.c. of an 18-hours' broth-culture of a not very virulent strain of B. Welchii. The second batch received a sublethal dose of T.H.N. (1 milligramme

in 0·4 c.c. of water). The third batch received both B. Welchii and 10 minutes later T H.N. The lethal dose of T.H.N. varies considerably with the temperature of the room in which the mice are kept. In our experimental conditions double the dose given, *i.e*, 2 milligrammes, was insufficient to kill the animals, while 2·5 milligrammes sometimes produced death. It may be added that if mice die as the result of the injection of T.H.N. they do so within a few hours (1–3 hours) after the injection, most frequently as the result of hæmorrhages into the lungs.

T.H.N. alone. Dose: 1 *mg. Site back.*	*Days after injection.* 1.	2.
Mouse No. 1.	Alive and well.	Alive and well.
„ 2.	do.	do.
„ 3.	do.	do.
„ 4.	do.	do.
Culture of B. Welchii. Dose: 0·25 *c.c.*		
Mouse No. 5. Injected subcutaneously.	Alive and well.	Alive and well.
„ 6. do.	do.	do.
„ 7. Injected intramuscularly.	do.	do.
„ 8. do.	do.	do.
0·25 *c.c. Culture of B. Welchii*+1 *mg. T.H.N.*		
Mouse No. 9. B. Welchii subcutaneously.	+	
„ 10. do.	+	
„ 11. B. Welchii intramuscularly.	Slightly ill.	Recovered.
„ 12. do.	+	

Effect of injection of T.H.N. on the action of the toxin of B. Welchii.

Experiment 13/8. Experiment similar to the preceding of 25/7, but toxin was injected instead of the living bacteria. The lethal dose of the toxin used was 0·5 c.c. The sublethal dose given in this experiment was 0·3 c.c. The toxin was injected subcutaneously in the flank at the same time as the T.H.N. was given subcutaneously on the back.

T.H.N. alone 1 *mg. Site back.*	*Next day.*
Mouse No. 1.	Alive and well.
„ 2.	do.
Welch toxin alone 0·3 *c.c. Site flank.*	
Mouse No. 5.	Alive and well.
„ 6.	do.
„ 7.	do.
„ 8.	do.
Welch toxin 0.3 *c.c. and T.H.N.* 1 *mg.*	
Mouse No. 9.	+
„ 10.	Alive and well.
„ 11.	+
„ 12.	+

The foregoing experiments demonstrate that the three conditions—exposure to cold, acidosis, and injection of T.H.N. have a prejudicial effect on the course of gas gangrene produced by B. Welchii, and that they are capable of transforming non-lethal doses of either the living organisms or of the toxin into lethal doses. The three conditions are very different in nature. But they have this in common that they put an extra strain on the functional activity of the suprarenal gland.* By adding this effect to the paralysing action of the toxin of B. Welchii on the gland they overtax the gland, which would otherwise have recovered from the effect of a non-lethal dose of the toxin. It is therefore probable that other conditions which put an "extra strain" on the gland, such as extensive hæmorrhage, or a sufficiently strong hyperthyroidism, will have a similar result. These conditions have not yet been studied by us experimentally in relation to gas gangrene, because it is more difficult to control them experimentally in such a way, that conditions exactly equal in degree can be produced in a number of animals.

The practical bearing which these observations have on the treatment of gas gangrene are obvious in the case of exposure to cold. Every case that is likely to develop the condition should be kept warm and dry and guarded against exposure and exhaustion, as soon as possible after it has been brought in.

The fact that the injection of acid may turn the scale in an infection with living bacteria from which the animal would otherwise recover throws light on an intrinsic feature of gas gangrene. Most of the anærobes concerned in gas gangrene are active acid producers when grown *in vitro*. It is therefore to be expected that acid is produced at the site of infection and absorbed by the blood-stream and tissue-fluids so that an acidosis is produced.

It is necessary here to define the term "acidosis," since it is used in different ways by different authors. The term acidosis is used here to indicate the formation and absorption by the tissue-fluids of acid substances, irrespective of the question whether the absolute hydrogen-ion

* The manner in which this is done differs in these three conditions, but it is not possible here to discuss these differences in detail without going into the question of the process of adrenalin formation and secretion, of which our knowledge is as yet very incomplete. The vague term "extra strain" is therefore used advisedly. But, although the way in which this effect is brought about is not yet clear in detail, the resultant effect is nevertheless very obvious and readily demonstrable histochemically.

concentration of the blood is thereby altered. It is a fact that by the experimental introduction of acid a pathological condition is produced in an animal although the alteration in the hydrogen-ion concentration in the blood may be too slight to be directly measurable by physical or chemical methods. The term acidosis applies to this condition and one of its features is the temporary partial depletion of adrenalin from the medulla of the suprarenal gland.

Wright (8) (9) has shown that in gas gangrene there is an acidosis both locally at the site of infection and also in the tissue-fluids. (The term "acidæmia" which he uses for the latter condition might be taken as implying that there is an alteration of the hydrogen-ion concentration of the blood. This, however, is not so, as Poulton (6) has found for *vibrion septique* and McIntosh for B. Welchii, so that the term "acidosis" seems preferable.)

Wright holds that the acidosis indicates a breaking down of the defences of the body against the invasion of the blood-fluids by bacteria from the site of the infection. This, however, cannot be the only explanation, because the introduction of acid makes an animal more susceptible not only to the action of the living bacteria but also to that of the toxin. Our observations show that the production of an acidosis is also a direct offensive mechanism on the part of the bacteria. By depleting the suprarenal medulla its effect adds itself to that of the toxin. This summation of effects accounts for the fact that the treatment of the acidosis by the injection of alkali is followed in some cases by such extraordinarily good results, while in other cases it appears to make no impression whatever on the fatal course of the disease. Both acidosis and toxæmia work in the same direction, and therefore measures directed against the acidosis alone are not likely to affect the course of the disease unless the toxæmia is comparatively weak. And conversely it becomes clear why the treatment with antitoxin in the later stages of gas gangrene is so frequently disappointing.

Although a sufficiently large number of clinical observations on this point have not yet been made, it is practically certain, from what is known of the behaviour of anærobes in culture, that a local acidosis occurs at the site of infection, which will lead to a general acidosis of varying degree, using the term acidosis in the sense defined above. The rapidity with which mice dying from gas gangrene pass into rigor mortis is strong confirmatory evidence. It is obvious therefore that measures against this condition should be taken right from the beginning by the administration of alkali, without waiting until the existence

of an acidosis has declared itself beyond doubt. When that latter point has been reached the effect on the suprarenal gland must already have taken place.

It is possible that another factor may contribute to the production of an acidosis. The toxin of B. Welchii has a distinct hæmolytic action. This leads to a reduction of the percentage of hæmoglobin (McIntosh, unpublished observations) and thus to an oxygen deficiency. It is known that the organism reacts to a want of oxygen by the production of an acidosis.

It is probable that one of the reasons why a mixed infection with a non-pathogenic organism like B. sporogenes adds to the severity of the condition in gas gangrene is to be found in the fact that B. sporogenes is also an active acid producer so that its presence will increase the acidosis. This is, however, not the only reason. For if a 48 hours' culture of B. sporogenes be filtered through a Berkefeldt filter and the resultant filtrate which is still alkaline to litmus be injected into animals which have received a non-lethal dose of toxin of B. Welchii, the animals will either die or at least suffer more severely and take several days to recover, although the filtrate itself would produce no visible effect. An explanation of this curious fact has not as yet been found. The following experiment illustrates this point :—

Experiment 2/8/18. 48 hours broth-culture of B. sporogenes filtered through a Berkefeldt filter. The filtrate was alkaline to litmus, slightly acid to phenolphthalein, 10 c.c. of the filtrate requiring 1·6 c.c. $\frac{N}{10}$ NaOH to make it neutral to phenolphthalein. Both the original filtrate and the filtrate made neutral to phenolphthalein were injected in doses of 1 c.c. on the back. Non-lethal doses of Welch toxin were injected in the flank.

		Result after		
		1 day.	**2 days.**	**3 days.**
Welch toxin alone (*Control*). Dose 0·5 c.c.				
Mouse No. 1.	Weight 13 g.	Ill.	(Killed.)	
„ 2.	„ 15 g.	Well.	Well.	Well.
Sporogenes Filtrate alone (*Control*). Dose 1 c.c.				
Mouse No. 3.	Weight 15 g.	Well.	Well: (killed).	
„ 4.	„ 13 g.	Well.	Well.	Well.
"Neutral" Sporogenes Filtrate alone (*Control*). Dose 1 c.c.				
Mouse No. 5.	Weight 14·5 g.	Well.	Well.	Well.
„ 6.	„ 13 g.	Well.	Well: (killed).	

Welch toxin+Sporogenes Filtrate.				Result after		
Dose 0·3 c.c.	Toxin+1 c.c. Filtrate.			1 day.	2 days.	3 days.
Mouse No.	7.	Weight	15 g.	Very ill: dying: killed.		
„	8.	„	14 g.	+		
„	9.	„	14·5 g.	Very ill.	Very ill.	+

Welch toxin+"Neutral" Sporogenes Filtrate.						
Dose 0·3 c.c.	Toxin+1 c.c. Filtrate.					
Mouse No.	10.	Weight	14 g.	Very ill: dying: killed.		
„	11.	„	14·5 g.	Very ill.	Very ill.	Very ill: (killed).
„	12.	„	14·5 g.	Very ill.	Very ill.	+

Results of P.M.—No. 7. Local reaction at site of injection of Welch toxin, slight. Abdominal organs: typical gas gangrene. No. 8. Same as No. 7. No. 10. Similar to No. 7, but reaction at site of injection more marked. Nos. 3 and 6. Normal. No. 1. Typical local reaction at site of injection has disappeared, beginning abscess formation, abdominal organs normal, except for a black clot in the bladder.

The action of T. H. N. in making an animal more susceptible to the effect of the toxin of B. Welchii or the inoculation of a broth-culture of this organism has not such a direct practical bearing on the treatment of gas gangrene as the other two factors of exposure to cold and acidosis. The value of the experiments with T.H.N. lies chiefly in the fact that its action is so sharply circumscribed and so clearly focussed on the suprarenal gland, and that the effect is very transient so that mice after the lapse of about 6 hours return to a normal condition.

A brief reference must also be made to the effect of anæsthetics upon the adrenal. In observations on the suprarenal gland of mice which have been subjected to anæsthesia by ether and by chloroform respectively it has been found that there is with both these substances a distinct depletion of the glands in an anæsthesia lasting for twenty minutes. This effect is more distinct with chloroform. Whether this is due to a direct effect of these two substances on the gland or whether it is due to the fall of the body temperature which occurred has not been determined. It must also be borne in mind that chloroform is likely to produce an acidosis as an after-effect, so that its use in gas gangrene appears to be strongly contra-indicated, as Wright has already emphasised. Our observations are only sufficient to indicate that the choice of a suitable anæsthetic in gas gangrene is a problem which deserves to be attended to by clinicians.

Morphine in doses sufficient to produce its pharmacological effect does not produce a definite effect on the suprarenal gland in mice. Nor does it in such doses transform a non-lethal dose of B. Welchii into a lethal one.

In this paper we have dealt entirely with the offensive mechanism by which the infection kills. The defensive mechanism by which the body protects itself against the infection will be dealt with in the following chapter II.

Summary.

The observations show that in the lethal issue of gas gangrene the exhaustion of the suprarenal gland plays an important if not a decisive part. It is brought about by a variety of conditions some of which are inherent in the infection while others are extraneous to it. The factors inherent in the infection are the production of a toxin which acts specifically on the gland, the production of an acidosis and the association with bacteria such as B. sporogenes, which by themselves are non-pathogenic. The extraneous factors are exposure to cold, physical exhaustion, hæmorrhage, anæsthesia, and perhaps also mental excitement. All these diverse conditions put an extra strain on the suprarenal gland, and by doing so add their effect to that of a toxin which by itself is of comparatively low toxicity. It is therefore of importance in the treatment of gas gangrene to watch the adrenal and guard it against exhaustion.

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II. On the Defensive Mechanism against the Bacteria of Gas Gangrene.

The defensive mechanism against the bacteria of gas gangrene is very efficient. It is so efficient that infection with the bacteria alone does not produce gas gangrene and does not even appear to produce any ill-health even in animals belonging to a susceptible species such as the mouse. In our first paper gas gangrene was produced experimentally by injecting small amounts (0·25 c.c.–0·5 c.c.) of a broth-culture of B. Welchii. Such a culture contains in addition to the living bacteria and the products of their metabolic activity (acids, etc.) also a toxin. If the toxin and the metabolites are removed from the bacteria by centrifugalisation and washing with normal saline, and if the washed bacteria are suspended in saline the resultant suspension when injected into mice in doses up to 1 c.c. does not produce gas gangrene. This negative result is obtained even if the volume of saline used for the suspension is only a half or a quarter of that of the original broth-culture so that a given volume of the suspension holds twice or four times the number of bacteria contained in the same volume of the original broth-culture.

The same negative result is obtained when a suspension made from a surface culture of B. Welchii is injected, and this holds good whether the injection is subcutaneous, intravenous or intramuscular.

These experiments have been repeated many times with young and old mice of different strains and with guinea-pigs, using different strains of B. Welchii. The results were uniformly negative except in one single case where an intramuscular injection produced gas gangrene in a mouse. Three other mice injected on the same day with the same suspension gave a negative result. It may be said therefore, that B. Welchii by itself is incapable of producing the specific disease. The same applies to *vibrion septique* and B. œdematiens. In order to free B. œdematiens completely from its toxins, which are more powerful than that of B. Welchii, it is necessary to heat the washed bacteria for half an hour at 80°, when spores are formed. These spores when injected into mice do not produce gas gangrene.

If now a few doses of a saline suspension of B. Welchii are added to a dose of Welch toxin, which by itself is insufficient even to make the animal ill, the mixture when injected produces a virulent gas gangrene which kills the animal in less than 24 hours. In such an experiment therefore, the two materials, of which the one by itself (the bacteria)

produces no effect at all in very much larger doses, while the other by itself does not produce any apparent ill-health in the restricted dose in which it is administered, will when mixed together produce a fulminating disease.

That the toxin itself is responsible for this effect and not the other metabolites also contained in the solution of toxin used, which was really only a broth-culture from which the bacteria had been removed by filtration, can be demonstrated by the following experiment. To some broth-culture is added antitoxin in doses sufficient to neutralise the toxin present. The antitoxin has no direct bactericidal action and does not affect the other metabolites. The mixture which therefore still contains the living bacteria and the other metabolites does not produce gas gangrene nor does it make the animal ill, even when large doses of the mixture are injected. It can also be shown that the action of the toxin is not specific. Thus a suspension of *vibrion septique* spores in non-lethal doses of Welch toxin will kill animals with the characteristic local lesion of *vibrion septique* gas gangrene. Similarly Tulloch has shown that Welch toxin and tetanus spores produce tetanus. Diphtheria toxin, which is non-toxic to mice but produces a slight local lesion when injected subcutaneously, will also sometimes elicit gas gangrene if injected together with *vibrion septique* spores or with washed B. Welchii. Even the toxin of a streptococcal abscess will often elicit gas gangrene from *vibrion septique* spores in mice, but not from B. Welchii.

The virulence of a mixture of toxin and a suspension of B. Welchii —meaning by virulence not only whether such a mixture kills or not but also the rapidity with which it produces death—depends not only on the absolute amount of toxin and the number of bacteria contained in it but also on the concentration of the toxin. Thus if to a fixed volume of toxin increasing amounts of a given saline suspension of bacteria are added, the mixture becomes at first more virulent, but when the volume of saline suspension added to the toxin reaches a point where the concentration of the latter is distinctly diminished, as for instance when equal volumes of toxin and suspension are mixed, the virulence of the mixture is again lessened. Thus in one experiment with a mixture of toxin and bacillary suspension in the proportion of 5 : 1, the animals showed signs of serious illness after 6 hours, and two out of three were dead within 20 hours after the injection. With a mixture of toxin and suspension in the proportion of 1 : 1, two out of three animals survived and the third animal did not die until the second day after the injection.

A similar result indicating the importance of the concentration of

toxin was obtained in the following experiment. A virulent broth-culture of B. Welchii was centrifugalised at a moderate speed. The bulk of the bacteria was thus collected at the bottom of the tube, but a few remained suspended in the supernatant fluid, as demonstrated by a film preparation. Injection of the supernatant fluid, which therefore represents a suspension of a comparatively small number of bacteria in toxin, killed mice rapidly in less than 20 hours in a dose of 0·25 c.c. If now equal volumes of a suspension in saline of the centrifugalised and washed bacteria and of the decanted supernatant fluid were mixed, the mixture which now contained in double the volume a much larger number of bacteria suspended in a diluted toxin was actually less virulent, since it took a longer time (more than 24 hours) to kill the animals.

An explanation of the observed facts is found when the site of inoculation is examined. There is a very obvious difference in the naked-eye appearance of the site of inoculation in the two conditions. When toxin has been injected with the bacteria there is the characteristic profuse hæmorrhagic œdema at the site of inoculation together with some necrosis of the subcutaneous tissue. After an injection of the bacteria alone there is only a slight œdema of the subcutaneous tissue, and if the injection has been made in the flank the inguinal lymph gland is distinctly swollen. After an injection of a suspension of washed B. Welchii the bacteria disappear from the site of injection partly by lysis and partly by phagocytosis. A film made from the site of inoculation 8 hours afterwards shows a large number of leucocytes containing ingested B. Welchii in various stages of disintegration. The phagocytosis is so active that many of the leucocytes are filled with the densely packed bacteria and only a few free bacteria can be seen. The same result is obtained when a mixture of a broth-culture and antitoxin has been injected. In preparations made from tissues fixed *in situ* the mass of injected bacteria is seen surrounded by leucocytes so that they are completely cut off from the tissue.

When a broth-culture of B. Welchii is injected the result during the first 5 or 6 hours is superficially similar. There is also a massive emigration of leucocytes towards the injected bacteria, surrounding them and ingesting them. But if this wall of leucocytes is examined under a high power most of the leucocytes show signs of disintegration and those lining the surface nearest the bacteria are actually undergoing lysis. The toxin destroys and dissolves the leucocytes, so that the wall of leucocytes which constitutes the defence gets eaten away and

dissolved. The bacteria are then enabled to proliferate freely throughout the tissues, and if the site of inoculation is examined the next day the tissues are overrun by masses of free B. Welchii and only few leucocytes are visible.

If only the later stages of the infection are examined the impression is necessarily formed that the toxin protects the bacteria against phagocytosis by exercising a negative chemiotactic influence against the leucocytes. It has been suggested that the toxin forms a zone of protection around the bacteria which the leucocytes cannot enter. The examination of the early stages shows that this impression is erroneous. The toxin does not paralyse the defensive mechanism of phagocytosis which continues to act, but it annuls it by killing and dissolving the leucocytes.

These observations show that normally the body is able to defend itself against a local infection with B. Welchii by lysis and by phagocytosis. This conclusion based on experiments *in vivo* is in accord with those arrived at by D'Este Emery (1) from *in vitro* experiments. The defences, which form a complete protection of the normal body against the bacteria alone, are overwhelmed by the toxin which thus acts locally by protecting the bacteria. From the moment, therefore, that a group of B. Welchii have surrounded themselves with toxin of sufficient concentration they are enabled to proliferate. They produce more toxin and extend the zone within which the defensive mechanism is overcome. The condition spreads thus with increasing rapidity: there is an absorption of toxin and of acids into the circulation, and the train of events is set in motion which has been studied in the previous paper. Gas gangrene may therefore be said to begin, not with the moment that the bacteria of gas gangrene are introduced, but with the moment that they have been allowed to form sufficient toxin to overcome the defensive mechanism of the body. The toxin of gas gangrene has thus two separate functions. At first it acts locally in the general manner of an aggressin and enables the bacteria to establish themselves in the body; later on it produces the specific toxæmia which, as shown in the first paper, together with other accessory factors contributes to the lethal issue. An understanding of this double function is of importance from the point of view of the administration of antitoxin. When antitoxin is given prophylactically, that is a few hours after the wound has been received, it ought to be injected locally, near but central to the wound. It can then counteract in its full strength the local action of any toxin that has been formed or may be formed, and by neutralising it may prevent the bacteria from establishing

themselves. If, on the other hand, the antitoxin is given therapeutically, that is when gas gangrene has developed and a toxæmia is threatening the life of the patient, then the antitoxin ought not to be applied locally, and is probably best given intravenously. For in such a case it is most important to combat first the general toxæmia so as to gain time to deal with the site of infection surgically.

In order to obtain some experimental evidence of the relative efficacy of antitoxin when given locally as compared with antitoxin acting through the general circulation, the following experiment was carried out. A number of mice received a fixed dose of a virulent broth-culture in the right flank and immediately afterwards varying doses of antitoxin on the back. Another set of mice received the same doses of broth-culture immediately after they had been mixed with varying doses of antitoxin. In the latter case, which represents an ideal case of local application, the smallest protective dose of antitoxin was found to be 0·003 c.c.; in the former case, in which the antitoxin acted through the general circulation, ten times the dose gave no protection and even a dose of 0·05 c.c. gave no complete protection, as one out of two animals died, while the other animal was very ill on the first day but recovered and survived.

It has been stated above that gas gangrene may be said to begin with the moment that the bacteria of gas gangrene have at one focus been allowed to form sufficient toxin to break down the defensive mechanism of the body. The observations recorded in this and the previous paper show how from this moment a vicious circle is initiated which leads with fulminating rapidity to a lethal issue. But the problem which now arises is: Why is this vicious circle ever initiated? In other words: Why does gas gangrene ever occur? Since inoculation with the bacteria of gas gangrene does not produce gas gangrene in the absence of the toxin, and since wounds are originally infected by the bacteria alone, gas gangrene ought never to occur. Clinical experience leads to the same problem. Almost every wound received in France was infected with B. Welchii. Only a small percentage of these wounds developed gas gangrene. Why gas gangrene developed in this small minority of cases is quite unknown. Although obviously this is a problem of the greatest practical importance, it has received little attention at the hands of previous workers. We believe we have found an answer to the question and shall deal with this in the next chapter.

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III. On a new Factor in the Mechanism of Bacterial Infection *.

Introduction.

The observations recorded in this paper were made in the course of investigations on gas gangrene. The work of McIntosh, of Weinberg and Séguin, and of other investigators has shown that the organisms chiefly responsible for the production of gas gangrene are the bacillus of Welch, the *vibrion septique*, and the Bacillus œdematiens. Small amounts of broth cultures of these organisms when injected into animals belonging to a susceptible species, *e.g.*, the mouse or the guinea-pig, produce a violent gas gangrene, and kill the animal within 24 hours.

It is known and was confirmed by us that suspensions in saline of B. Welchii and of *vibrion septique* from a surface culture are practically non-pathogenic ; half a cubic centimetre of a dense suspension of these organisms can be injected into a mouse or guinea-pig subcutaneously or intramuscularly without producing gas gangrene and, indeed, without producing any signs of ill-health. The same result is obtained if broth cultures of B. Welchii or of *vibrion septique,* which contain toxins in addition to the bacteria, are centrifuged, and the bacteria, after having been washed free from adherent toxin, are suspended in saline and injected. In the case of B. œdematiens, the toxin is so potent that it is not easy to remove the last traces of toxin by washing, and it is necessary to destroy the last traces of toxin by heating the washed bacteria to 80° C. for half-an-hour, when spores are formed. These spores, when suspended in saline and injected, are again non-pathogenic. Very occasionally it does happen that gas gangrene develops after the injection of detoxicated bacteria or their spores, but such an event is quite exceptional and apparently accidental. With the detoxicated B. Welchii, for instance, we have observed gas gangrene to occur only once out of a large number of experiments on more than a hundred animals. But if these "detoxicated" bacteria or spores are again mixed with a dose of toxin too small to produce an effect by itself, gas gangrene develops regularly and kills the animal.

Further investigations, which will be published separately, showed that the bulk of the detoxicated bacteria when injected undergoes lysis, while some of the bacteria are taken up by phagocytes and slowly undergo disintegration within the phagocytes, but that these two

* This chapter is a reprint of a paper published in the 'Proceedings of the Royal Society,' 1919, B., vol. 90.

processes, by means of which the animal defends itself against the infecting bacteria, do not take place when the bacteria are injected together with toxin. It is clear, therefore, that the toxin paralyses the defensive mechanism of the animal. But it also follows that this defensive mechanism is normally so efficient that the formation of toxin in a concentration sufficient to produce this paralysing action is prevented.

We were thus led up to the problem : Why does gas gangrene ever occur in man ? Since the defences of the body against the bacteria of gas gangrene are so efficient, the infection of wounds with these bacteria is not in itself an adequate explanation of the occurrence of gas gangrene. And clinical experience leads up to the same problem. Very many wounds, especially those received on the Western Front, were infected with the bacillus of Welch, but only a very small percentage of those infected wounds developed gas gangrene.

It is clear that a new factor, extrinsic to the infection, enters here, and it was in the search for this factor that the phenomenon recorded in this paper was observed and found to have a more general significance.

The small incidence of gas gangrene in wounds infected with the bacteria of gas gangrene is, of course, well known. The explanation generally given is that, owing to the special conditions obtaining in war wounds, the latter offer an exceptionally favourable nidus for the growth of these bacteria. Thus, interference with the circulation, the presence of large masses of dead and lacerated muscle, the presence of cloth, dirt or foreign bodies generally, have all been suggested as factors capable of eliciting gas gangrene. But clinical experience shows that many wounds, in which these factors are undoubtedly present, do not develop gas gangrene, and that there are no clinical features of a wound pathognomonic of gas gangrene. And experiment completely fails to give any support to the nidus theory. Splinters of wood, pieces of muscle, of paper, wool, cotton-wool, and of khaki cloth, have been soaked in a suspension of B. Welchii, and then introduced underneath the skin of a mouse without ever producing gas gangrene. Even the minced muscle from a meat broth culture of B. Welchii does not produce gas gangrene if introduced subcutaneously after having been freed from toxin by washing with saline. Nor was it found possible to produce gas gangrene by cutting the femoral artery and injecting a suspension of B. Welchii into the leg or into the hæmatoma, or by injecting suspensions of B. Welchii, together with staphylococci, streptococci, B. coli, B. proteus, or B. sporogenes. The injection of

lactic acid in varying concentrations, together with the detoxicated bacteria of gas gangrene, has also failed to produce gas gangrene, even although an extensive sore was frequently produced as the result of the injection of the lactic acid.

The negative results have been reported in detail because they demonstrate clearly how difficult it is to elicit the pathogenic action of the bacteria of gas gangrene or their spores. In this respect they resemble the bacterium of tetanus. We are informed by Dr. Tulloch that, in his experience, it is not possible to induce tetanus regularly and with certainty after infection with the spores by any of the means given in the literature and tested by him. As the negative results of the experiments given above accumulated, the conviction grew that in normal animals, in which the experimental conditions can be controlled and kept constant, an adequate explanation of the relation between these bacteria and the specific disease with which they are associated cannot be deduced from the occasional and apparently accidental production of gas gangrene or of tetanus, as the case may be, which sometimes occurs, but must be based on a definite factor, by which these conditions can be elicited from the bacteria or their spores with certainty and at will. Such a factor was eventually found in injections of small doses of a soluble ionisable calcium salt. The severity of the trauma which can be inflicted on animals infected with the detoxicated bacteria or their spores without eliciting from them the specific disease, stands in striking contrast to the phenomenon which will now be described.

Calcium Salts as an Accessory Factor in Gas Gangrene and Tetanus.

Doses of 2·5 mgrm. of calcium chloride, when injected subcutaneously into mice of 10–15 grm. weight, together with a suspension of a virulent strain of B. Welchii or of *vibrion septique*, will produce a violent gas gangrene in every case. The same dose when injected with a suspension of the tetanus bacillus or its spores will produce tetanus. Larger doses up to 5 mgrm. have the same effect. With mice the dose of calcium chloride cannot safely be increased above 10 mgrm., since with doses of such magnitude the toxic action of calcium salts comes into play. Doses of 10 mgrm. are, as a rule, without any ill effect for normal mice of 15–20 grm. weight. Sometimes, however, even with such a dose the animals are ill a few hours after the injection and die within 24 hours. With smaller doses the effect becomes at first

irregular, and if the dose is further diminished fails to appear. The smallest dose with which it has been possible to cause gas gangrene with the spores of *vibrion septique* has been 0·5 mgrm. of calcium nitrate. For guinea-pigs of 250 grm. weight the minimal dose necessary to produce this effect in every case is larger, namely, 5 mgrm. of calcium chloride. With B. œdematiens experiments were made only with spores, for the reasons already given, and for these larger doses are necessary to elicit their pathogenic action, namely, 5 mgrm. for a mouse and 10 mgrm. for a guinea-pig. In all our experiments the solutions of calcium salts were used in a strength of 1 per cent. or 2 per cent. It is possible that the dose of calcium salt necessary to produce the effect may vary with the concentration. This point has not yet been tested by us.

For the sake of convenience and brevity it is advisable to designate this new phenomenon by a new name. For reasons which will be given below, and which are based on the mechanism by which the phenomenon is produced, the terms "kataphylaxis," or "defence-rupture," will be used in this paper to describe it. The smallest dose of a calcium salt necessary to elicit the specific disease from the detoxicated bacteria in every case is called the "minimal rupturing dose." Smaller doses which are still capable of eliciting the specific disease, but are not capable of doing so in every case, are called "subminimal rupturing doses." In fixing these doses it is of course assumed that a virulent strain of the specific bacteria is being used. The density of the bacterial suspensions can apparently be varied within wide limits without affecting the results. We have worked, as a rule, with a suspension of such a density that large print is just readable through it in a test-tube. But we have also used denser and more dilute suspensions with practically identical results.

Calcium nitrate and calcium acetate have the same effect as calcium chloride. The insoluble calcium carbonate has no rupturing action. Similar doses of the chlorides of potassium, sodium, ammonium, magnesium, and strontium * have no effect when injected together with a suspension of B. Welchii.

B. sporogenes, when injected together with calcium chloride, does

* [*Note added April* 4.—We have since found that with *vibrion septique*, the pathogenic properties of which are more readily elicited than those of *B. Welchii*, strontium will produce gas gangrene, if sufficiently large doses (5 mgrm.—10 mgrm.) are given. But, even then, a positive result is not obtained in every animal, as is the case with *B. Welchii*.]

not produce gas gangrene, and does not even make the animal ill. It may be recalled that even in broth cultures this organism is non-pathogenic, if present alone.

The rupturing action of calcium chloride is abolished by sodium citrate. As an illustration the following experiment will be given. A number of mixtures of 2 per cent. solutions of calcium chloride and sodium citrate were made up to a volume of 2 c.c. with water. Two drops of a dense freshly prepared suspension of B. Welchii in saline were added to these mixtures ; 0·5 c.c. of each of these mixtures were injected subcutaneously into batches of three mice. The results were as follows :—

Batch.	$CaCl_2$ solution 2 per cent.	Na citrate solution 2 per cent.	H_2O.	Dose of $CaCl_2$ injected.	Mouse 1.	Mouse 2.	Mouse 3.
	c.c.	c.c.	c.c.	mgrm.			
A	1	—	1	5	+	+	+
B	1	0·5	0·5	5	+	+	+
C	1	1	—	5	0	0	0
D	0·5	—	1·5	2·5	+	+	+
E	0·5	0·5	1	2·5	0	0	0
F	0·5	1·5	—	2·5	0	0	0

In the Tables + means "died," 0 means "alive and well."

All the mice in batches A, B, and D developed gas gangrene and died within 20 hours after the injection, except one animal in batch D, which died within 48 hours. All the animals in batches C, E, and F remained alive and well for three days after the injection.

By means of a similar experiment it has been possible to demonstrate an antagonism between Ca ions and Mg ions with reference to the production of gas gangrene from B. Welchii. 0·2 c.c. of a suspension of these bacteria was added to mixtures of equimolecular solutions of $MgCl_2$ and $CaCl_2$ in varying proportions. These mixtures were then injected subcutaneously into batches of four mice each. In each batch two mice received a "rupturing dose" of calcium chloride, *i. e.*, a dose which contained 2·75 mgrm. of $CaCl_2$, two mice received a "subminimal rupturing dose," containing 1·4 mgrm. of $CaCl_2$, *i. e.*, a dose which does not elicit gas gangrene in every case. The results are as follows :—

Batch.	$CaCl_2$ solution M/5.	$MgCl_2$ solution M/5.	H_2O.	Dose of $CaCl_2$ injected.	Result after 3 days.	
					Mouse 1.	Mouse 2.
	c.c.	c.c.	c.c.	mgrm.		
A	0·5	—	1·5	2·8	+	+
B				1·4	+	+
C	0·5	0·5	1	2·8	+	+
D				1·4	0	0
E	0·5	1	0·5	2·8	+	+
F				1·4	0	0
G	0·5	1·5	—	2·8	+	0
H				1·4	0	0

The experiment shows a distinct protective action of the magnesium salt against a subminimal rupturing dose of the calcium salt, when tested with B. Welchii.

In all the experiments mentioned so far, the bacteria or their spores have been injected suspended in the various salt solutions. This direct contact between the bacteria and the calcium salts gives the most favourable conditions for the phenomenon which we have described, but it is not essential. It has been possible to produce gas gangrene in mice by injecting the spores of *vibrion septique* and calcium salts either at the same site at different times, or secondly, at the same time at different sites, the injection of calcium salts either preceding or following that of the spores. Similarly, tetanus has been produced by injecting the spores and the calcium salt at different sites at the same time and also by injecting calcium salts and tetanus spores at the same site but at different times, the injection of spores also either preceding or following that of the calcium salts. In such an experiment, for instance, no tetanus occurred for seven days in mice which had received an injection of tetanus spores. But, when, on the seventh day, calcium chloride was injected tetanus developed on the following day.

These experiments, in which the injections of calcium salts and of bacteria were separated in time or in space, throw light on the mechanism by which the peculiar effect of calcium salts, with which this paper deals, is brought about. A detailed consideration of these experiments will be given below in dealing with the probable explanation of the phenomenon.

On the Etiology of Gas Gangrene and of Tetanus.

We have referred in the introduction to the fact that infection with the bacteria of gas gangrene and of tetanus is not in itself an adequate explanation of the specific diseases produced by these bacteria, and that it was necessary to postulate the existence of an accessory factor. The question which naturally suggests itself is whether the phenomenon which we have described in this paper represents this accessory factor.

It has always been realised that both tetanus and gas gangrene are diseases particularly associated with earth, and, in fact, these diseases can be produced by injecting emulsions of earth into mice or guinea-pigs. The explanation which is generally accepted is that the soil contains the spores of the specific bacteria. No specific action is credited to the earth itself ; it is supposed to act as a foreign body like many other things, such as pieces of cloth, or splinters of wood, the presence of which is assumed to create an exceptionally favourable nidus for the growth of the bacteria. As stated in the introduction we have failed to find any experimental evidence which would support the nidus theory. Vaillard*, who investigated in great detail the relation of the contamination of the wound with earth to the etiology of tetanus, and whose observations as to the inadequacy of the nidus theory agree in many respects with ours, recognised that this contamination exercises a specific influence. But he ascribed this specific factor to the presence of other micro-organisms in the earth.

Of the various foreign bodies tested by us, emulsions of earth were the only ones which frequently, although not invariably, elicited gas gangrene when injected together with the detoxicated bacteria of gas gangrene or their spores. That the effect of earth is not due to its mechanical action but to a chemical constituent of the earth was demonstrated by the following experiment: A sample of earth was taken which, when autoclaved and made into an emulsion, would elicit gas gangrene from the spores of *vibrion septique*. The watery extract of such an emulsion, after having been filtered through filter paper, autoclaved and tested for its sterility, was just as capable of eliciting gas gangrene as the original emulsion. It could be shown, moreover, by qualitative chemical tests, that this earth extract contained calcium salts and that it lost its power to elicit gas gangrene when these salts had been removed by precipitation with sodium carbonate. We are not however prepared to state that the calcium salts in the soil are the

* Vaillard et Rouget, 'Annales de l'Institut Pasteur,' vol. 6, p. 385 (1892).

only chemical constituents which are responsible for this phenomenon, for some extracts of earth which were capable of eliciting gas gangrene from the spores of *vibrion septique* contained only traces of calcium salts. It seems probable that some other chemical substance present in these extracts, which also forms an insoluble carbonate, is also capable of producing the kataphylactic phenomenon, and may be even more powerful in this respect than calcium salts. Further investigations on this point are being carried out.

It is of interest to note that samples of earth taken from the surface may fail to show this effect when a sample from the same locality about 6 inches below the surface will give a positive result. It has also been found that samples from different localities differ in their activity, and that samples taken from the same locality and from the same depth may show differences at different times of the year, *i. e.*, according to the treatment, such as "liming," which the soil has received. These differences may not exhibit themselves qualitatively by the presence or absence of the power to elicit gas gangrene or tetanus, but quantitatively by the doses of earth extract or emulsion necessary to produce this effect. These observations account satisfactorily for the curious fact that the occurrence of gas gangrene on the Western Front was very "patchy." It varied with the locality in which the wounds had been received, and was relatively infrequent in certain localities, even although the wounds were infected with the bacteria of gas gangrene.

There can be little doubt that the presence of certain simple chemical constituents of the soil which have the property of producing the kataphylactic phenomenon is responsible for the occurrence of gas gangrene and of tetanus. This statement does not imply that every case of gas gangrene and of tetanus can be accounted for in this way. Experimentally, mice infected with detoxicated B. Welchii and exposed to cold have occasionally developed gas gangrene. As a rule, gas gangrene does not develop in mice exposed to cold either before or after the injection of a suspension of B. Welchii. But out of 16 mice, two animals, in which the exposure to cold had been exceptionally severe so that the animals were already ill when they received the injection of B. Welchii, did develop gas gangrene. The experiments with cold are complicated by the fact that mice frequently develop an enteritis as the result of a severe exposure to cold. The general depression of the vitality of an animal which has received such a severe exposure to cold may be reasonably assumed to involve also the processes of lysis and phagocytosis which constitute the defensive mechanism of the infected

animal against the infected bacteria, and thus account for the development of gas gangrene in the two positive experiments. In the human subject, where such an expression may be the result not only of exposure to cold but also to shock, it will probably be responsible also for the development of gas gangrene in some cases. But it cannot be looked upon as the only or even most frequent exciting cause, since gas gangrene develops in men who, apart from the wound, are in good health, are not in a state of shock, and have not been exposed to cold.

There is another way in which tetanus and gas gangrene can be produced experimentally in animals infected with the detoxicated bacteria without making use of the phenomenon of defence rupture. Tulloch * has shown that tetanus spores will produce tetanus in guinea-pigs if they are injected together with a non-lethal dose of the toxin of B. Welchii. We have found that gas gangrene can be produced in mice if the detoxicated B. Welchii are injected together with diphtheria toxin, which happens to be non-lethal for mice but has a transient local action. The explanation of this fact is probably to be found in the aggressin-like nature of the toxins. We have pointed out in the introduction that the toxin of B. Welchii acts at first by paralysing the defensive mechanism of lysis and phagocytosis by which the animal defends itself against infection with the detoxicated B. Welchii, and diphtheria toxin has probably a similar effect in mice. It seems possible, therefore, that the presence of a non-specific and non-lethal toxin with an aggressin-like action may have to be considered as a factor in the causation of gas gangrene or of tetanus. As stated in the introduction, we have not been able to obtain experimental evidence in support of this view in the case of gas gangrene by injecting the bacteria of gas gangrene together with other bacteria which are likely to form concomitant infections in wounds. And even in the production of tetanus from the spores by means of the toxin of B. Welchii, it must be remembered that this does not represent the effect produced by a concomitant infection with the bacilli of Welch. For such an infection does not, as we have seen, lead to the production of toxin sufficient to paralyse the defensive mechanism. It still requires to be demonstrated that tetanus will result when the spores of tetanus are injected together with the detoxicated bacteria of Welch.

The preceding considerations emphasise the importance of the contamination of the wound with earth from the point of view of the

* Tulloch, 'British Medical Journal,' June 1, 1918.

etiology of gas gangrene and of tetanus. For this contamination carries into the wound not only the infecting bacteria, but also frequently the chemical constituents of the soil capable of producing the phenomenon of defence-rupture. But there is also some experimental evidence to show that tetanus and gas gangrene may sometimes develop even without the intervention of the phenomenon.

On the Mode of Action of Calcium Salts.

Two possible explanations suggest themselves at once : the calcium salts may produce their effect either by making the bacteria more virulent, or by making the animal more sensitive to the action of the bacteria. Both these possibilities were tested experimentally.

In order to see whether there is a direct action on the bacteria, B. Welchii freed from toxin were suspended in a solution of calcium chloride and incubated for three hours. The suspension was then centrifuged, and the bacteria after washing with saline suspended in a sodium chloride solution, and the suspension injected into mice. No effect was produced.

The same negative result was obtained when B. Welchii were incubated for 20 hours in a broth culture to which some calcium chloride solution had been added. A suspension of B. Welchii in saline prepared from such a culture was incapable of producing gas gangrene. It is clear, therefore, that calcium salts do not act directly on the bacteria in such a way as to render them capable of producing gas gangrene by themselves in the absence of a toxin. Nor does the addition of calcium chloride to a broth culture increase the amount of toxin produced. This was demonstrated both for B. Welchii and for *vibrion septique*. It was however noted that the rate of growth of B. Welchii and of *vibrion septique* was much more rapid after the addition of calcium chloride. It was further found that a culture of B. Welchii or *vibrion septique*, obtained by inoculating meat broth with fragments of breast muscle from pigeons which had been inoculated four hours previously with a broth culture of these organisms to which calcium chloride had been added, was not more virulent than a culture from a pigeon which had been infected with broth cultures alone of these bacteria.

It is clear, therefore, that the action of calcium chloride is not due to a change in the essential properties of the bacteria so as to make them individually more virulent. This conclusion is confirmed by the observation, to which reference has already been made, that a direct

contact between the bacteria and calcium salts is not essential for the production of the kataphylactic effect. There remains the other alternative, that the effect of calcium salts might be to make the animals more sensitive to the action of the bacteria. Now it is known that the bacteria of gas gangrene and of tetanus owe their pathogenic effect to the formation of a toxin. The correctness of the second alternative can therefore be tested by determining whether the injection of calcium chloride diminishes the minimal lethal dose of these toxins. This test was applied to the toxin of B. Welchii. It was found that the injection of calcium salts did not make an animal more sensitive to the action of this toxin. What, then, is the explanation of the phenomenon described in this paper if it cannot be attributed either to an increased sensitiveness of the infected animal or to an increased virulence on the part of the infecting bacteria? A partial answer to this question is given by the experiments, in which the injections of calcium salts and of bacteria were separated in time or in space.

Gas gangrene will result if *vibrion septique* spores are injected into an animal which some hours or days previously has received an injection of calcium salts. This result is obtained most readily if the two materials are injected into the same site, and in that case the interval between the two injections may extend over several days if a large dose of calcium salt has been given. Thus in two different experiments the following results were obtained: 2·5 mgrm. Ca salt given two hours before *vibrion septique* spores: injected into same site into eight mice. Result.—All mice dead within 24 hours. 10 mgrm. Ca salt given three days before *vibrion septique* spores: injected into same site into nine mice. Result.—All mice dead within 24 hours. Even if the two materials are inoculated in two different sites, gas gangrene may follow, although not in every case.

The following experiment may be given to illustrate the result of separating the sites and times of injecting the bacteria and the rupturing substance. In this experiment 36 mice, in 12 batches of 3 mice each, received at the same time injections of calcium nitrate in varying doses. At two different intervals of time—2 and 24 hours afterwards—the mice received injections of a suspension of *vibrion septique* spores, half the number of animals being injected subcutaneously at the same site (right flank), the other half being injected subcutaneously on the back. The results were as follows:—

Gas gangrene from *vibrion septique*. Effect of separating Time and Site of Injections of Bacteria and of Calcium Salt.

Batch.	No. of mouse.	Dose of Ca nitrate.	Interval of time.	Site.	Result after 24 hours.
		mgrm.	hours.		
A	1	2·5	1¼	Same	+
	2				+
	3				+
B	4	2·5	1¼	Different	0
	5				0
	6				0
C	7	2·5	20	Same	+
	8				+
	9				+
D	10	2·5	20	Different	0
	11				0
	12				0
E	13	5	1¼	Same	+
	14				+
	15				+
F	16	5	1¼	Different	0
	17				+
	18				+
G	19	5	20	Same	+
	20				+
	21				+
H	22	5	20	Different	0
	23				0
	24				0
J	25	10	1¼	Same	+
	26				+
	27				+
K	28	10	1¼	Different	+
	29				0
	30				0
L	31	10	20	Same	+
	32				+
M	33	10	20	Different	0
	34				0
Control	35	Spores alone	—	—	0
	36				0

This experiment shows that a minimal rupturing dose will elicit gas gangrene in every case from the spores of *vibrion septique*, even when the latter are injected after an interval of 20 hours into the same site. When different sites are used, however, the production of gas gangrene

becomes more difficult, and it is necessary to use larger doses of the calcium salt and to inject the spores within a short time after the calcium salts. *Post-mortem* examinations were made of one dead mouse in each batch. All showed the typical lesion of *vibrion septique* gas gangrene in the right flank, and this was the case even in the three mice where the spores had been injected on the back. Only one of these three mice had the typical lesion also on the back ; the two others only showed a slight serous exudate, such as is found in normal mice after the injection of spores. This remarkable fact will be referred to again below.

Similar experiments were carried out with a suspension of B. Welchii. With these bacteria the dose of calcium salt has to be increased to 5 mgrm. if an interval of time is allowed to elapse before the injection of the bacteria, and this interval of time within which gas gangrene can be produced is limited to a few hours. After an interval of 24 hours, we have not been able to produce gas gangrene even with a dose of 10 mgrm. of calcium salt. Doses of 10 mgrm. of calcium salt are necessary when different sites are used. Under these conditions, we have observed two different courses which the infection may take. Either, as in the case of *vibrion septique*, the typical lesion of B. Welchii gas gangrene is produced at the site of injection of the calcium salt, and may be completely absent from the site where the bacterial suspension was introduced ; or there is no distinct localised gas gangrene lesion, but a B. Welchii septicæmia results, in which the hæmolytic action of the infection seems to predominate. For in such animals B. Welchii can be seen in films made from the heart blood, the liver and kidney are very pale instead of showing the intense congestion of a typical B. Welchii gas gangrene, and the blood is very poor in hæmoglobin.

Experiments with spores of *B. tetani* (Type II of Dr. Tulloch) gave essentially the same results as those with B. Welchii and the spores of *vibrion septique*. As the Table shows, it was possible to elicit tetanus by injecting the spores $2\frac{1}{2}$ hours after the injection of even the minimal rupturing dose of the calcium salt, when the spores were injected into the same site as the calcium salt. But, when different sites were used, tetanus did not occur even with the biggest dose of calcium salt. Even with an interval of 24 hours tetanus was produced, but then it was delayed and occurred only with larger doses of calcium salt and using the same site. It must be noted, however, that for the experiments with a 24-hours' interval, a different and older preparation of spores was used, which appeared to have lost some of its virulence, since the

spores did not produce tetanus in every case when injected together with a rupturing dose of a calcium salt.

The details of the experiment are given in the following Table. It is perhaps necessary to point out that this experiment is quite different from those mentioned in an earlier part of this paper, in which tetanus spores were injected first and then calcium salts.

Tetanus. Effect of separating Time and Site of Injections of Bacteria and of Calcium Salt.

Batch.	No. of mouse.	Dose of Ca nitrate.	Interval of time.	Site.	Result after 1 2 3 4 days.
		mgrm.	hours.		
A	1	2·5	$2\frac{1}{2}$	Same	0 0 0 0
	2				0 + — —
B	3	2·5	$2\frac{1}{2}$	Different	0 0 0 0
	4				0 0 0 0
C	5	5	$2\frac{1}{2}$	Same	0 + — —
	6				0 + — —
D	7	5	$2\frac{1}{2}$	Different	0 0 0 0
	8				0 0 0 0
E	9	10	$2\frac{1}{2}$	Same	0 + — —
	10				0 + — —
F	11	10	$2\frac{1}{2}$	Different	0 0 0 0
	12				0 0 0 0
G	13	5	24	Same	0 0 0 +
	14				0 0 0 0
H	15	5	24	Different	0 0 0 0
	16				0 0 0 0
J	17	10	24	Same	0 + — —
	18				0 0 + —
K	19	10	24	Different	0 0 0 0
	20				0 0 0 0
Control	21	Spores alone	—	—	0 0 0 0
	22				0 0 0 0
	23				0 0 0 0
	24				0 0 0 0
	25				0 0 0 0
	26				0 0 0 0

0 means alive and well with absence of symptoms of tetanus. + means died with symptoms of tetanus.

All these experiments point to the conclusion that the injection of calcium salts produces at the site of injection a local change in the tissues of the animal since the specific disease is most easily elicited when the calcium salt and the bacteria are injected into the same site. It does not appear reasonable to suppose that this local effect is due to the actual presence of calcium salts at the site of injection. Since the effect is produced only by soluble, ionisable calcium salts, it would be necessary to assume that such a salt could remain deposited at the site of injection for a day or even three days. Moreover, if that were so, it ought to be possible to counteract their effect by injecting sodium citrate together with the bacterial suspension. That, however, we have been unable to do. In one experiment, for instance, eight mice received an injection of 2·5 mgrm. of calcium chloride in the right flank. Two hours afterwards four of the mice received a suspension of *vibron septique* spores in saline in the right flank, while the other four received a suspension of these spores in 2 per cent. sodium citrate solution, also in the right flank. All the eight mice were dead within 24 hours with the typical local lesion of gas gangrene. Similar experiments have been carried out with the same result. Sodium citrate only protects when it is mixed with the calcium salt before it is injected into the animal.

One must conclude, therefore, that calcium salts produce a local change in the tissues at the site of injection. This conclusion is confirmed by the fact that it is much more difficult to produce gas gangrene and tetanus when the bacteria and the calcium salts are injected at different sites, than when they are injected at different times. The local change produced in the tissues by calcium salts persists for a considerable time, and has, as the ultimate result, the formation in the animal of a place of diminished resistance against the infecting bacteria. The most striking confirmation of this conception is furnished by the result of the experiments in which gas gangrene occurred after injection of the calcium salt and bacterial suspension at different sites. For in these experiments the fact was observed that the typical local lesion of gas gangrene—an intense hæmorrhagic œdema—occurs always at the site of injection of the calcium salt, while the site where the bacteria have been injected does not show, as a rule, any macroscopic evidence of gas gangrene. Films made from the two sites present this difference very strikingly (see fig. 1); the film from the site of injection of B. Welchii shows active lysis, as indicated by the presence of bacterial *débris*, and active phagocytosis with intracellular digestion of the

engulfed bacteria. Only a few bacteria can still be seen lying free and apparently intact, but many of these have become gram-negative. The picture is practically identical with that given by a normal mouse which has received a suspension of detoxicated bacteria, and which is defending itself successfully against the infection. But the film from the site of injection of the calcium salt presents the picture typical of an animal which has developed gas gangrene by, let us say, the injection of the bacteria together with toxin. The whole field is covered with densely packed bacteria which are almost all gram-positive. Only very few leucocytes can be seen, and none of these show any phagocytosis.

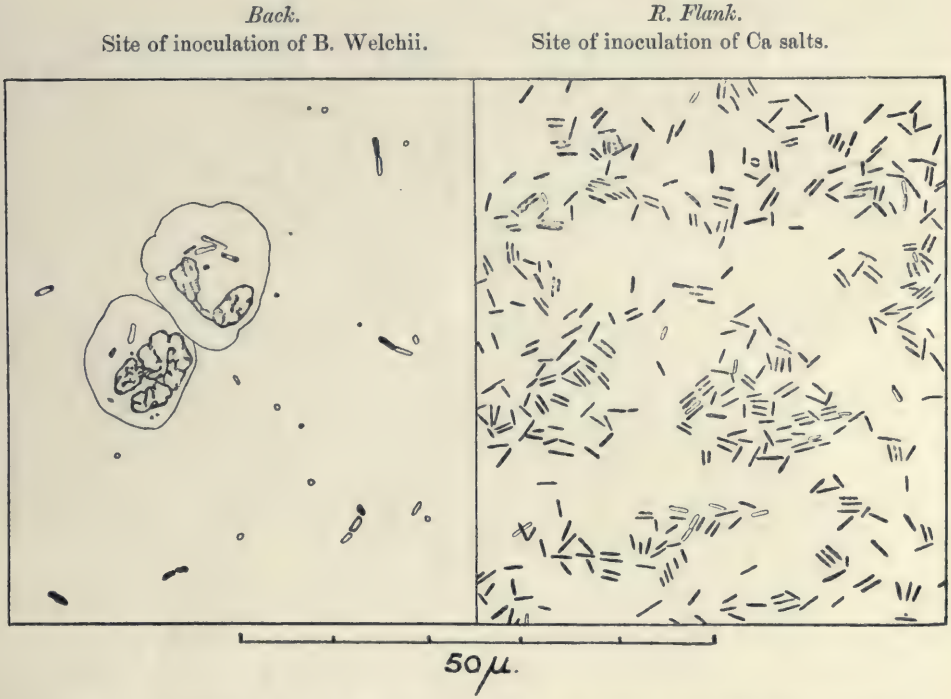

FIG. 1.—Films from the back and right flank of a mouse which developed gas gangrene and died 24 hours after having received first 10 mgrm. of $CaCl_2$ in the right flank, and 2 hours later a suspension of B. Welchii in saline on the back. Stained with gram and neutral red. Gram-negative staining is indicated by merely outlining the bacteria.

Both macroscopically and microscopically the picture presented by the right flank is that of an animal which has succumbed to the B. Welchii infection; the picture presented by the back that of an animal which is successfully defending itself against such an infection.

This apparently parodoxical result becomes clear in the light of the conception that the calcium salts produce at the site of their injection a local change in the tissues which has as its ultimate effect a local breaking down of the defensive mechanism. This conception of the mechanism is expressed by the terms "defence rupture" or "kataphylaxis," with which we have designated this phenomenon.

When calcium salts and bacteria are injected together it is quite possible that this effect may be assisted by other factors. There may be a direct favouring effect of the calcium salts on the rate of growth of the bacteria, such as has been observed *in vitro*. It is conceivable too that calcium salts may directly interfere with phagocytosis and lysis, and experiments *in vitro* will have to be carried out in order to test this point. But these factors, even admitting for the sake of the argument that calcium salts did produce these effects *in vivo*, could only be accessory factors under the special experimental conditions which involve the actual presence of calcium salts. When a separation of the sites or times of the injections is made, the actual presence of calcium salts can be excluded, but the phenomenon still makes its appearance.

Summary.

The bacteria of gas gangrene (B. Welchii, *vibrion septique*, and B. œdematiens) and of tetanus, when completely freed from their toxins, either by washing or by heating to 80° C. for half-an-hour, so that spores are formed, do not produce the specific disease when injected into a mouse or a guinea-pig. The normal animal disposes of the bacteria mainly by lysis and partly also by phagocytosis, and this defensive mechanism is so efficient as to render these bacteria non-pathogenic when injected by themselves.

If a small dose of a soluble ionisable calcium salt is injected together with the bacteria or their spores, the specific disease is elicited in a very virulent form. The chlorides of sodium, potassium, ammonium, strontium and magnesium, when injected together with B. Welchii, are not capable of producing gas gangrene.

A direct contact between the bacteria and the calcium salt is not essential. The phenomenon will occur if the bacterial suspension and the calcium salt are injected at different times into the same site, or into different sites at the same time or at different times.

From these experiments and other experimental evidence the conclusion is drawn that calcium salts, when injected subcutaneously,

produce a local change in the tissues at the site of injection. The effect of this change is to bring about a local breaking down of the defensive mechanism against the bacteria of gas gangrene and tetanus. The terms "kataphylaxis" or "defence rupture" are proposed to designate this new phenomenon.

Sterile watery extracts of earth are capable of producing this phenomenon. They may owe this property in many cases entirely to the presence of calcium salts, but there is evidence that in some cases the extracts of earth owe their rupturing action to the presence of another chemical substance or substances which have not yet been identified.

The bearing of these observations on the etiology of gas gangrene and tetanus is discussed.

The figure is by Mr. W. Pilgrim, the laboratory draughtsman. We wish to express our great indebtedness to Dr. H. Henry, Dr. J. McIntosh, Dr. R. A. O'Brien, Miss Muriel Robertson, and Captain W. J. Tulloch, R.A.M.C., for their readiness in assisting our investigations by supplying us with information and material.

IV. Further Observations on the Mechanism of Bacterial Infection.

Introductory.

In the preceding chapter on the mechanism of bacterial infection, the conclusion was arrived at that infection with the bacteria of gas gangrene and of tetanus will lead to the production of the specific disease or intoxication if there is also in the tissues of the animal a lesion of a special nature at the site of infection. This special lesion can be induced experimentally with great regularity by the injection of definite amounts of soluble ionisable calcium salts. We did not, at the time when the preceding paper was sent to the Royal Society for publication, investigate the nature of the lesion, nor did we even know whether such a lesion could be demonstrated either macroscopically or microscopically. It was also predicted that other substances besides calcium salts would be found to be capable of producing this phenomenon of defence rupture. And thirdly, it was suggested that the phenomenon was likely to have a general significance and to play a part in other bacterial infections, besides those of gas gangrene and tetanus. These three aspects of the problem have been investigated and the results are given in the present chapter.

Lesion produced by Calcium Salts.

The subcutaneous injection of 2·5 mgrm. to 5 mgrm. of a soluble ionisable calcium salt into a mouse of about 15 grm. weight produces an extensive lesion, which is not only visible to the naked eye, but is even palpable. After 24 hours there is an extensive slightly opaque gelatinous exudate at the site of injection which presumably consists of clotted plasma and clotted lymph. The blood-vessels in the subcutaneous tissue are congested, the abdominal wall at the site of injection also shows vascular dilatation and a pinkish discoloration of the muscles. The exudate is slowly absorbed with the formation of adhesions. Sometimes a small ulcer appears after about seven days. This is probably due either to an accidental infection or to the injection having been made too superficially. Microscopically the following changes can be seen 24 hours after the injection: the arterioles, capillaries, and veins are dilated, and many of the vessels are filled with densely packed red blood corpuscles, so that the contents appear solid and without plasma (Pl. VI. figs. 2 & 3; Pl. VIII. fig. 4; Pl. VII. fig. 5). White thrombi can be seen in some of the capillaries; they may either narrow the lumen or they may completely occlude it. The lymph vessels are widely dilated. There is a massive emigration of leucocytes, chiefly of the polymorphonuclear type, and occasionally a diapedesis of red blood corpuscles. The intima often shows degenerative changes, the endothelial cells being swollen or partly detached, and sometimes the endothelial lining appears to have disappeared. The muscle fibres in the immediate neighbourhood of the lesion are degenerated with loss of transverse striation and formation of hyaline masses, and are invaded by leucocytes. The lymph glands in the neighbourhood of the injection, for instance the inguinal gland if the injection has been made in the flank, show a distinct change. The fine blood capillaries in the gland are widely dilated and filled with densely packed blood corpuscles without any intervening plasma, and thus present a picture similar to that of the vessels in the subcutaneous tissue. The lymph sinuses of the gland are also widely dilated and contain besides lymphocytes a small number of endothelial cells which have become detached. Some sinuses are filled with a network of fibrin.

The lesion produced by the subcutaneous injection of calcium salts shows the typical features of an aseptic inflammation. It may be looked upon as a simultaneous toxic action of calcium salts on the various tissues. Or if one assumes that the calcium salts have a primary

toxic effect on the endothelial cells, most, if not all, of the other features of the lesion can be interpreted as resulting from this primary lesion. Thus, in the case of necrosis of the muscle fibres it is doubtful whether this is due to a direct toxic action of the calcium salts or whether it is secondary to the interference with the circulation. The fact that the necrotic change is confined to comparatively few muscle fibres, although a volume of half a cubic centimetre of the calcium salt solution has been injected, which in the mouse covers a comparatively large area of the abdominal wall, suggests that the second alternative is the correct one.

None of these effects are produced when a mixture of calcium nitrate and sodium citrate is injected. It will be recalled that we showed in the preceding communication that such a mixture does not produce the phenomenon of defence rupture.

It is interesting to note that the local action of Welch-toxin resembles in many respects that of the calcium salts. It also produces damage to the endothelial wall with the production of thrombi, emigration of leucocytes, and degenerative changes in muscle fibres.

Other Substances capable of producing the Phenomenon of Defence Rupture.

Such substances are (1) Strontium ; (2) Distilled water in relatively large amounts, viz., 1 c.c. or more for a mouse of 10–15 grammes weight ; (3) Suspension colloids, both electropositively and electronegatively charged, such as colloidal silicic acid, colloidal iron hydroxide, colliodal palladium, colloidal gold ; (4) Emulsion colloids. The only one which has been tested so far is gelatine, which has given a positive result.

The colloidal silicic acid was prepared in the laboratory by adding hydrochloric acid to a solution of sodium silicate and dialysing the mixture against distilled water. The final solution had a strength of 0·7 per cent. It was neutral to methyl orange. Colloidal gold and colloidal palladium were commercial preparations, colloidal iron hydrooxide was the B. P. preparation. Gelatine was used in a 5 per cent. solution and in a 0·1 per cent. solution. None of these substances produce their effect with the same regularity as calcium salts, even when the doses are increased. At the same time the amounts necessary to produce the effect are not necessarily large as compared with doses of calcium salts. Thus 0·5 c.c. of a 0·1 per cent. solution of gelatine injected together with *vibrion septique* spores produced gas gangrene

in two out of three mice. The following experiment with silicic acid may be given as an illustration of the irregularity of the action :—

Mice No.	Dose of Silicic Acid.	Dose of *vibrion septique* suspension.	Result next day.
1–6	0·5 c.c.	—	All alive and well.
7	0·1 c.c.	0·25 c.c.	+
8	0·1 c.c.		+
9	0·1 c.c.		+
10	0·25 c.c.		+
11	0·25 c.c.		Alive and well.
12	0·25 c.c.		do.
13–18	—	0·5 c.c.	All alive and well.

It is to be noted that mice Nos. 13–18 received double the dose of organisms that was sufficient to produce gas gangrene in mice Nos. 7–10 when given together with silicic acid.

The various types and strains of bacteria differ in the readiness with which the specific disease can be elicited from them by the phenomenon of defence rupture. We pointed out in the preceding paper that the minimal rupturing dose of calcium salts requires to be higher in the case of B. œdematiens than in the case of either B. Welchii or of *vibrion septique*. With the substances given in this paper a similar difference exists between *vibrion septique* on the one hand and B. Welchii on the other. *Vibrion septique* is the organism from which the specific disease is elicited most readily. Thus strontium salts, which, as stated in the preceding chapter III., fail to elicit gas gangrene from B. Welchii, will produce gas gangrene when injected together with *vibrion septique* spores. But even then the disease is not produced in every case, although large doses of 10 mgrms. were given. The same applies to some of the other substances mentioned in this paper. We have failed to produce gas gangrene from the washed bacilli of Welch by injecting them together with gelatine, colloidal gold, palladium, and iron hydroxide. It must remain doubtful, however, whether this difference can be referred to biological differences between the two types of organisms. It is possible that this difference may disappear with a more virulent strain of B. Welchii than the one we have used in these experiments, since our strain gave evidence of having become attenuated.

The dose of distilled water necessary to produce the effect varies considerably with the conditions under which it is injected. If a few drops of a saline suspension of *vibrion septique* spores are added to the distilled water the large dose of 1 c.c. is necessary to produce defence rupture. The proportions used in these experiments were one drop of the saline suspension to 1 c.c. of water. But if the spores are washed free of salt solution and then suspended in water, this suspension kills in a smaller dose (0·25 c.c. to 0·5 c c.). The rupturing action of a dilute salt solution (about 0·05 per cent.) is therefore weaker than that of pure distilled water.

The lesion produced by these substances has been studied for water, strontium salts, gelatine and colloidal silicic acid. It is in its essential features identical with the lesion produced by calcium salts, although not so severe, and shows formation of thrombi, stasis of blood-vessels, emigration of leucocytes, dilatation of lymphatics and the pouring out of an exudate which is only slowly absorbed (Pl. VII. fig. 6 ; Pl. VIII. fig. 7).

The Effect on the Tissues of Substances not capable of producing Defence Rupture.

The effects produced by the injection of normal salt solution, 2 per cent. magnesium nitrate solution, and the mixture of calcium nitrate and sodium citrate have been studied. The obvious difference is the absence of any gross macroscopic lesion. There is no exudate of clotted plasma and lymph, but only a very slight serous exudate. Microscopically there is no thrombosis. There is an emigration of leucocytes. The effect produced by the three substances mentioned above differs in degree. With magnesium salts the emigration of leucocytes is abundant and there is some evidence of stasis with diapedesis of red blood corpuscles. This has not been observed with the other two substances. Magnesium sometimes produces also a lesion of a few muscle fibres in which there is a proliferation of the sarcolemmal nuclei and an invasion of the fibres by leucocytes (Pl. VIII. fig. 8). The lesion is, however, neither as extensive nor as severe as that produced by calcium salts. The capillaries in the lymph gland are only very slightly dilated if at all. But the lymph sinuses at the hilum show a very marked dilatation, and in the case of the magnesium salts there is a very active swelling and proliferation of the endothelial cells lining these sinuses. These cells are actively phagocytic and many of them become detached and can be seen lying free in the lumen of the sinus.

On the Action of Lactic Acid.

It is stated, and it appears to be generally accepted, that lactic acid is capable of eliciting tetanus in animals which have received injections of detoxicated tetanus spores. This statement was originally made by Vaillard. Besson, experimenting with the spores of *vibrion septique*, and Leclainche and Vallée with B. chauvœi, obtained similar results. All these investigators found in addition, that when detoxicated spores are injected with fine sand the specific disease is produced. The general conclusion drawn was, that any substance which protects the injected spores from phagocytosis will enable the spores to germinate and produce the disease.

Relying on this statement we began our attempts to produce gas gangrene from the detoxicated bacteria of gas gangrene by injecting solutions of lactic acid of varying strength either together with the bacteria or separately. In the latter case the acid was injected either before or after the bacteria and either at the same site or at different sites. As stated in our previous paper all these experiments, which were numerous, were negative. Lactic acid even in dilute solutions produces frequently an extensive sore at the site of injection and may even kill the animal. But in spite of the severe lesion the specific disease is not elicited from the bacteria of gas gangrene, as evidenced by the complete absence of the characteristic local lesion.

Our experiments with tetanus spores, which were less numerous, gave equally negative results. The much more extensive experience of Dr. Tulloch with tetanus spores has, as stated in our previous paper, led to the same negative results.

It may be pointed out in passing that it is easier to decide whether the specific disease has been produced in experiments with the organisms of gas gangrene than with tetanus. In the former case there is the unmistakeable evidence of the characteristic local lesion, so that even in an animal which has died overnight it is possible to state with certainty whether the animal has died from the specific disease or from the lactic acid alone.

It does not appear to be realised that lactic acid is a very toxic substance. In a concentration of 33 per cent. lactic acid kills in a dose of 0·2 c.c. In a concentration of 10 per cent. 0·5 c.c. is fatal. With smaller doses or with a more dilute solution (5 per cent. of lactic acid) an intense local lesion is produced: the skin is digested and sometimes

all the abdominal wall ; even the abdominal organs near the site of injection, the liver for instance, may show a patch of necrosis.

It was thought possible that lactic acid might be capable of inducing the germination of spores and that the bacteria thus formed might then be dealt with by lysis and phagocytosis. In order to test this point an experiment was carried out in which a series of mice received *vibrion septique* spores. Half the animals received an injection of 5 mg. of calcium nitrate, the other half was injected with 0·5 c.c. of 5 per cent. lactic acid. One mouse of each group was killed every hour and a film made from the site of injection. After 5 hours the mice which had been injected with calcium showed the first appearance of a red œdema. Those injected with lactic acid showed a blackening of the tissues at the site of injection and beginning digestion of the skin. The films showed only spores. After 6 hours the calcium mice showed a distinct hæmorrhagic œdema and the films showed numerous free bacteria but hardly any spores. The lactic acid mice in which the skin had by that time been digested away gave on examination of films only spores.

The interest which attaches to these negative results lies in the fact that it illustrates in a very striking manner that the phenomenon of defence rupture is not dependent on the severity or the extent of the lesion, both being much greater with lactic acid than with calcium, but on the specific nature of the lesion.

The experimental basis for the contention that lactic acid is capable of eliciting tetanus from the detoxicated spores of tetanus appears to be very slender. It depends apparently entirely on a casual statement, in which no details are given of the strength of the acid, or the amount injected, or the number of animals used. We have also made a number of experiments in which *vibrion septique*, intimately mixed with sterile sand, has been injected into mice and guinea-pigs, but our results have been uniformly negative.

Is the Phenomenon of Defence Rupture due to the Production of Conditions favourable to Anaerobiosis ?

The production of thrombosis and stasis by the substances producing defence rupture suggests as a possible explanation of this phenomenon the production of conditions favourable to anaerobiosis. The following facts show that this explanation is not correct. In the first place as will be shown below for aerobic bacteria the same specific tissue lesion is an important factor in determining the course of the infection.

In gas gangrene, as it occurs in war, the causative anaerobes invade the blood from which they may be isolated by blood culture. This is particularly true of *vibrion septique*. Experimentally we have shown that this occurs in animals. In mice injected with a broth-culture of a mildly pathogenic strain of B. Welchii the organism may be recovered from the blood 1½ hours after injection of the culture. The heart-blood of animals dead of gas gangrene almost invariably contains the infecting bacillus. Moreover, the intravenous injection of a mixture of silicic acid and *vibrion septique* spores in mice leads to death, whilst an equal volume of silicic acid alone does not kill.

In such an experiment three mice received an injection of 0·25 of colloidal silicic acid containing *vibrion septique* spores. All these animals died during the next day. Control experiments with silicic acid alone showed that the intravenous injection of this substance has no apparent effect. Apart from the site of injection, where the tail becomes gangrenous, there was no macroscopic local lesion and since there was no formation of gas the condition should not be called gas gangrene. The blood was watery and poor in hæmoglobin, probably as the result of the action of a hæmolysin which was formed. Here, too, the bacteria could be seen in blood films made from the heart-blood.

It may be a matter for argument whether or not the circulating blood presents conditions of anærobiosis, and whether indeed such conditions are necessary for so-called anærobes to develop in the living animal. But since these organisms can grow in the circulating blood, it is difficult to argue that the deficient oxygenation of a circumscribed area, which is one of the results of the thrombosis produced by calcium salts and other substances producing defence rupture, is the determining factor which is responsible for this phenomenon.

There is also the fact mentioned in our previous paper that cutting of the femoral artery, which must necessarily produce a profound deficiency in the oxygenation of the tissues of the leg, does not in itself make the limb a more suitable soil for the experimental production of gas gangrene by the detoxicated bacteria. The negative results obtained with lactic acid where extensive necrosis of tissue occurs, offer arguments leading to the same conclusion.

Is the Presence of Muscle Tissue necessary to evoke the Pathogenic Action of the Bacteria of Gas Gangrene?

The widely held view embodied in this question is based chiefly on clinical grounds. Gas gangrene is most frequently seen in wounds

of the arms and legs, which necessarily involve masses of muscle. Clinically the diagnosis of gas gangrene rests partly on the characteristic change in the muscles and on the formation of gas which arises from the glycogen of the muscles as the result of the fermentative action of the bacteria. Moreover, from a study of the morbid histology of the disease M^{c}Nee has been led to the conclusion that a necrosis of muscle is a condition preliminary to the spread of the bacterial infection. But experimental investigation negatives this conclusion. First, the mere presence of a muscle lesion, whether produced by the knife or by such chemical substances as lactic acid, does not elicit gas gangrene from the detoxicated bacteria. Secondly, the experiment mentioned in the preceding paragraph, in which the pathogenic nature of the bacteria manifested itself in a septicæmia after an intravenous injection of the bacteria together with colloidal silicic acid, clearly excludes the presence of muscle tissue, whether normal or injured, as an essential factor. And thirdly, the examination of early stages of the infection when produced experimentally by the subcutaneous injection of the detoxicated bacteria and calcium salts shows that during the first six hours there is an active proliferation of the bacteria throughout the areolar tissue and not necessarily involving any muscle fibres. It has been stated above that the injection of calcium salts produces *inter alia* a degeneration of muscle fibres in a circumscribed area. But the proliferation of the bacteria during the first few hours after the injection of the bacteria does not necessarily occur in the neighbourhood of these injured muscle fibres. If the conception of gas gangrene is limited by the clinical picture, it can of course be argued that the presence of muscle is necessary for the disease, since without it neither gas formation nor gangrene will be visible. But if the question is taken in its more general bacteriological aspect, it must be concluded that the pathogenic action of the bacteria can manifest itself in the absence of muscle tissue.

The Phenomenon of Defence Rupture in Streptococcal Infections.

In order to determine whether this phenomenon was generally applicable and particularly whether it was applicable to infection by aerobic bacteria, the course of a streptococcal infection produced in mice by the subcutaneous injection of saline suspensions was studied. For the first strain of Streptococcus pyogenes used we are indebted to Dr. C. H. Browning.

The subcutaneous injection in mice of $\frac{1}{4}$ to $\frac{1}{2}$ c.c. of a saline suspension made from a 24 hours' agar-culture produces after 24 hours a sharply circumscribed collection of pus with a diameter of about 0·3 to 0·5 cm. (Pl. IX. fig. 9). This hardly ever involves the abdominal or thoracic wall, nor does the skin become affected as a rule. The subcutaneous blood-vessels in the neighbourhood of the collection of pus are slightly dilated, and the inguinal lymph gland on the side of the injected flank is enlarged. The collection of pus diminishes in size during the next few days and after 5 or 6 days may be completely absorbed. The animals look well and do not in appearance give any evidence of ill-health. Microscopically the typical picture of an abscess is presented: a central necrotic mass of dead leucocytes surrounded by a thick wall of living leucocytes containing streptococci. Numerous chains of free streptococci are visible also in the necrotic area.

When a calcium salt is injected either together with the streptococcal suspension or 24 hours before, the animals appear ill. If the animal is killed after 24 hours and the skin is reflected, a spreading abscess is seen which may cover the entire flank of the animal and which always involves the abdominal wall (Pl. IX. fig. 9). The blood-vessels are widely dilated. The skin is usually involved and there is frequently ulceration. If no ulceration occurs the abscess is never absorbed during the first week. Microscopically the picture is that of an intense and widespread cellulitis. Streptococci can be seen proliferating everywhere throughout both the subcutaneous tissue and the muscle fibres of the abdominal wall. They can be seen in the blood-vessels and lymphatics. Large accumulations of living leucocytes are scattered throughout the tissues exhibiting an active phagocytosis. Sometimes there is a long continuous line of leucocytes, the arrangement showing clearly an attempt to limit the spread of the infection. But the bacteria have broken through this line which is now surrounded by streptococci on either side. There are, of course, also extensive areas of necrotic tissue (dead leucocytes) in which streptococci are lying free.

With a different strain of streptococci, for which we are indebted to Dr. J. McIntosh, even more striking results were obtained. A batch of four mice received a suspension in sodium chloride of streptococci from a 48 hours' culture, another batch of four mice were injected with a suspension of streptococci in a 2 per cent. calcium nitrate solution. All the mice of the latter batch looked ill and two of them died on the

third day after the injection, one died on the fourth day and the last surviving mouse was then killed, looking ill. None of the mice injected with the sodium chloride suspension of streptococci appeared to be obviously ill at any time after the injection. They were killed on the fourth day. The post-mortem examination showed in two mice of this control batch one small sharply circumscribed abscess in the subcutaneous tissue at the site of injection, in the other two there were two such small sharply defined abscesses. The skin and abdominal wall were not involved. The internal organs were normal to the naked eye. In the three mice of the calcium batch which had died there was a widespread collection of diffluent pus covering the whole right flank and part of the abdominal wall, and involving the skin so that an ulcer was formed. The subcutaneous tissue over the whole side of the animal was deeply congested as were the kidneys and adrenals. The liver, kidney, and spleen when examined microscopically showed the presence of an intense septicæmia. Streptococci were seen lying in the larger blood vessels as well as in the finest capillaries. The liver cells showed cloudy swelling. The fourth animal of this batch which was killed showed the same extensive spreading abscess involving the abdominal wall and the skin ulcerating through it. But the pus was not so diffluent and there were no macroscopic changes in the internal organs.

There does not appear to be any difference in the virulence of the streptococci in the control animal and in the animals which have received a calcium salt. For if some of the pus is inoculated into fresh animals the pus from a control animal is as capable of producing a fresh abscess as the pus from the animal in which defence rupture has been induced. The injection of streptococci together with magnesium nitrate produces the same result as the injection of a saline suspension. Sometimes the absorption of the streptococcal pus was completed even more rapidly than in the control animals (Pl. IX. fig. 9).

On the early Stages of the Infection by the Bacteria of Gas Gangrene.

In order to study the early stages of the infection in gas gangrene detoxicated B. Welchii were injected subcutaneously both alone and together with or after a dose of calcium nitrate. The animals were killed at hourly intervals during the first 8 hours and the subcutaneous tissues fixed in situ in Formol-salt solution or in Zenker's fixative.

When this is done it is found that the course of infection presents in its initial stages essentially the same features as that followed in

infection by streptococci. When the bacteria of Welch are suspended in sodium chloride or magnesium nitrate solution and injected, they become rapidly surrounded by leucocytes which cut off the mass of injected bacteria from the tissue and rapidly dispose of them, so that after 24–48 hours few free bacteria are left (Pl. X. fig. 10). If the bacteria are injected together with a calcium salt they do not become surrounded by leucocytes. They proliferate freely throughout the areolar tissue ; leucocytes can be seen either singly or in aggregated masses amongst the bacteria and actively phagocyting them (Pl. VIII. fig. 11). But the mechanism by which the leucocytes can surround the bacteria and thus cut them off is evidently upset, so that the leucocytes become overwhelmed by action of the toxin produced by the rapidly proliferating bacteria. In the later stages (7–8 hours) many of the leucocytes lying amidst the bacteria are undergoing degeneration and dissolution by the toxin (see p. 37).

General Discussion and Summary.

In trying to give an explanation of the action of calcium salts and other substances having a similar action in reference to the mechanism of bacterial infection, it is possible now to arrive at certain definite conclusions.

It is obvious from the observations recorded above that the phenomenon of defence rupture cannot be due either to an absence of leucocytes or to an inhibitory action of the calcium salts on the process of phagocytosis. The presence of a muscle lesion or the necrosis of tissues generally can also be excluded. Similarly the production of anaerobic conditions is not in itself the determining factor. It may perhaps be responsible for the germination of the spores, but we have not been able to obtain any experimental evidence in support of such a view. We have failed to find conditions which would enable the spores to germinate without also producing the phenomenon of defence rupture. If this could be done it would be a matter of considerable practical importance, since it would then be possible to do away with the danger of delayed tetanus or delayed gas gangrene.

One factor which determines the course of an infection is related to the mechanism by which the leucocytes are first guided to the focus of infection so as to surround it and then, having dealt with it, are removed again and enabled to dispose of the injected bacteria. This mechanism is evidently upset by the injection of calcium salts and substances having a similar rupturing action. From the lesions in the

blood-vessels and lymphatics indicating a stasis and the production of a clot of lymph and plasma it seems reasonable to conclude, that the disturbance of the vascular and lymphatic drainage of the tissues is responsible for the disturbance of the mechanism which presides over the movements of the leucocytes towards the focus of infection and away from it. The inability of the tissue fluids and the anti-bacterial substances contained therein to permeate tissues filled with clotted lymph and plasma may also play a part.

Further investigations will be necessary to work out in detail the various factors which contribute to the phenomenon of defence rupture. The general significance of these observations may be summed up as foilows :—In many bacterial infections the presence of virulent bacteria in the body is not necessarily followed by the specific disease. A second factor is necessary which has been identified as a tissue lesion of a certain well defined type. This lesion can be produced experimentally by the introduction from without of a number of substances. And in the cases of gas gangrene and tetanus an introduction from without of such substances due to the contamination of the wound with earth is actually a decisive factor in the etiology of the disease. But the nature of the lesion makes it probable that it may occur also as the result of pathological processes affecting the vascular and lymphatic endothelium. Such a lesion, when it occurs, would enable a bacterial infection to spread through the tissues instead of remaining confined to a small focus where it would eventually be overcome.

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Besson : Ann. de l'Inst. Pasteur, ix. 1895, p. 179.
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[*Sixth Scientific Report of the Imperial Cancer Research Fund*, 1919.]

CELLULAR CHANGES IN CARTILAGE GRAFTS.

By J. A. MURRAY.

(Plate XI.)

The use of grafts of costal cartilage to replace extensive defects in plastic operations on the face, raises the important question of the ultimate fate of the transplanted tissue. The investigation, the results of which are given here, was undertaken at the suggestion of Prof. Arthur Keith who handed over the human material supplied to him by Major H. D. Gillies. I am indebted to both for the ample way in which my requirements in the supply and preservation of material have been met.

The results of the transplantation of hyaline cartilage have frequently been the subject of investigation from the early years of the 19th century onwards. The literature of the subject is fully reviewed in Prof. Marchand's volume on Wound-healing (2) in Billroth and Luecke's "Deutsche Chirurgie." While the transplantation of embryonic cartilage usually leads to the formation of considerable nodules by proliferation of the cartilage cells, most authors are agreed that this does not occur when cartilage from young or adult individuals is used. The pieces acquire a close organic union with their surroundings and persist apparently unaltered for a long time. Grafts of embryonic cartilage on the contrary, after having attained their maximum size, show degenerative changes in cells and matrix and are absorbed.

The density of the matrix of cartilage hinders rapid penetration of most fixing fluids with consequent unequal preservation of superficial and deeper parts. The best results have been obtained by fixing in 10 per cent. Formol salt solution for at least 24 hours (the pieces may remain in the solution indefinitely). Sections 10–15 microns thick are cut with the sliding microtome after imbedding in Salkind's lead gum (3), and transferred to 1 per cent. sodium chloride solution for 10 minutes to one hour. Slides are prepared beforehand by coating with a thin layer of 1 per cent. gelatine and allowing to dry. The prepared slide is immersed in the salt solution, and the sections are arranged in order

with a smooth-pointed glass rod. The superfluous fluid is drained off and a wetted cigarette paper carefully lowered over the sections. Firm pressure with several layers of filter paper makes the sections adhere to the gelatine and absorbs the excess of moisture. The cigarette paper is withdrawn and the slide exposed to formalin vapour for a few minutes. It is then transferred to 10 per cent. formol salt solution for 5 minutes, 5 per cent. acetic acid 10–20 minutes, washed in water to remove the lead-gum, and stained. The most useful stain for cartilage cells has been found to be Hollande's chloro-carmine (1) followed by Sudan, to stain fat. The sections are mounted in glycerine jelly.

In a mesial longitudinal section of a normal rib cartilage from a young mouse prepared in this way, the perichondrium appears as a laminated fibrillar layer in which the flattened nuclei between the fibres show as deeply stained rods. Passing inwards the fibrillation gives place to a homogeneous zone of hyaline material containing compressed cells with a finely granular homogeneous protoplasm and flattened nuclei very similar to those in the perichondrium. The transition is gradual and the collagenous fibrils merge without a break into the homogeneous ground substance of the hyaline cartilage. The dense staining reaction of the latter with basic dyes fades at the margin and in haematoxylin-van Gieson preparations for example there is a zone, almost unstained, intervening between the pale blue cartilage and the red stained collagen fibrils of the perichondrium.

In the deeper layers the nuclei of the cartilage cells become vesicular and the protoplasm vacuolated. Some of these vacuoles contain fat, at first as small droplets, which coalesce to form larger drops in the more centrally placed cells. In addition, in appropriately fixed preparations, fine granules and larger masses of glycogen are seen, and these are represented in the ordinary preparations by large clear vacuoles in the protoplasm. As the fat droplets increase in size they cause rounded depressions in the nuclei. When the droplets coalesce into one large globule, appearances identical with those of ordinary adipose tissue may be produced. It is important to recognise that this may occur in normal cartilage, and is not necessarily to be interpreted, when seen, as fatty degeneration. In the central parts of the costal cartilage nuclear changes occur of a more far-reaching kind. The nuclei extrude a great part of their fluid contents and become irregular in contour and stain deeply. The change is indistinguishable from that usually called pyknosis by pathologists. Many nuclei also present a delicate furrow-like indentation opposite the greatest accumulation of

cytoplasm, and ring-nuclei occur. These appearances are encountered in exaggerated degree in cartilage grafts and will be referred to later. Away from the epiphyseal zones mitotic cell division is extremely rare even in the cartilages of young growing animals.

These nuclear changes are accompanied by alterations in the matrix in calcified cartilage. There is a fragmention into irregularly cubical masses, each containing a number of cells, separated by fibrous strands. In a costal cartilage from an old mouse, acicular refractile crystals soluble in dilute acetic acid were seen in these fibrous septa. The hyaline matrix also showed the granular calcification which is usual in human calcified cartilages.

Grafts are conveniently designated as autologus, homologous, or heterologous, according as donor and recipient are the same individual, are separate individuals of the same species, or belong to different species. The human material placed at my disposal consisted of autologous and homologous grafts of varying ages. To assist in the interpretation of the cell changes, a series of homologous grafts of mouse rib cartilages were made and examined at intervals of a few days for the first two weeks, and thereafter at longer intervals up to 6 months. In addition, two autologous grafts were made in young mice of the costo-chondral junction and examined, one after a month, and the other after 6 months. The appearances did not differ appreciably from those to be described in the homologous grafts.

Apart from a pronounced fatty degeneration in the perichondrial cells (fig. 1) there is no change to be observed in such a graft after two days. The outer layers of the perichondrium apparently degenerate, and are replaced by new connective tissue from the host; but there is in all probability a survival and recovery of the inner layer next the cartilage, as this is found in the same state as in a normal cartilage, in grafts four days old.

The hyaline matrix remains unaltered in appearance and staining reactions for many weeks. It is only in grafts several months old that fibrillation begins, and still later before finely granular calcification takes place. Irregular fragmentation into roughly cubical masses was encountered in the autologous graft in the mouse killed 6 months after transplantation. The process is practically the same as that described in the calcified rib cartilage of an old mouse. The degenerative process had evidently been accelerated, for the cartilages of the mouse which had been left in situ were still hyaline and translucent.

The behaviour of the cartilage cells varies considerably according to

their distance from the perichondrium. Fig. 2, from a homologous graft in a mouse killed 4 weeks after transplantation, illustrates the appearances in the long period of quiescence which precedes the advanced degenerative changes. The flattened cells of the surface layers retain their normal appearance in the oldest grafts examined. In this region also the matrix maintains its glassy normal appearance unaltered. Passing inwards from the surface the cells still show the vacuolation and accumulation of fat droplets described for a normal cartilage. The nuclei, however, show very striking changes. As soon as the vacuolated layer is reached, a narrow groove appears on one side of the nucleus, and gradually increases in depth until the nucleus is divided into two parts connected by a delicate thread of chromatin. In some cells the connecting strand remains at both sides of the nucleus, and the groove widens out so that a ring nucleus results. Further in, cells with two nuclei occur, and the conclusion is almost inevitable that this is a later stage of the same process, which must therefore be regarded as an amitotic nuclear division. Naturally it is impossible to prove that this process has been in play when two cells are seen, as frequently occurs, in one capsule of matrix. It is possible to argue that mitotic division, taking place rapidly at long intervals, and therefore not observed, is responsible for the nuclear divisions followed by cell division. It is very unlikely that this is so, as in no nucleus in a graft has a condensation of chromosomes been observed such as precedes and follows mitosis. The only proliferative appearances met with in the nuclei of autologous and homologous grafts from the human subject are of the amitotic kind described.

In one human autologous graft examined, amitotic nuclear divisions were accompanied by other appearances in the cells, of a clearly degenerative character. This graft was removed six months after transplantation. Figs. 3–6 illustrate very clear examples of amitosis from this specimen. In figs. 3 & 7 there is in addition an irregular system of fibrils radiating from a finely granular area of denser protoplasm. Two such centres with a connecting achromatic spindle are shown from another cell in fig. 6. In fig. 4 the achromatic fibrils form a broad band passing across the cell from one side to the other. Two features are remarkable in these fibrillar systems : the coarseness of the fibrils and the absence of centrosomes. They are evidently of the same nature as the achromatic attraction or spindle fibres which play a part in mitotic cell division, but they do not bear any constant relation to the nuclear changes which accompany them. Taken in

conjunction with their coarseness and the absence of centrosomes, they indicate a thoroughgoing disorganization of the internal mechanism of the cells in which they occur. The remaining two figures (figs. 8 & 9) show the final stage in cell degradation met with in this graft. In these the nucleus, or nuclei, have undergone fragmentation into a number of large and fine masses. In fig. 9 the process has gone further than in fig. 8, and the protoplasm is uniformly filled with a fine chromatin dust.

The conclusion is drawn from a consideration of the human and animal cartilage grafts examined, that the maintenance of the physical properties of hyaline cartilage after grafting, depends on the survival of the cartilage cells. Processes of the same kind as those which occur in undisturbed cartilage as senile changes, take place in cartilage grafts, and sometimes more rapidly.

There is, therefore, a distinct probability that calcification and secondary absorptive processes may set in, in human cartilage protheses, sooner than might have been expected. Should this expectation unfortunately be realized, it may be necessary to devise some artificial substitute for cartilage.

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(2) F. Marchand, Wundheilung, Stuttgart, 1901.
(3) J. Salkind, C. R. Soc. Biol. 1916, vol. lxxix, p. 811.

[*Sixth Scientific Report of the Imperial Cancer Research Fund*, 1919.]

HISTOLOGICAL METHODS OF DEMONSTRATING BACTERIA IN TISSUES.

By J. A. MURRAY.

(Plate XII.)

The shortcomings of the methods in use to demonstrate bacteria in sections made themselves felt in the course of the work of reporting on pathological specimens for Medical Officers in the Army. Gram's method is only applicable to Gram-positive organisms and leaves very little choice in the matter of counterstains, so that duplicate preparations must be made for ordinary histological purposes. The method of staining Gram-negative as well as Gram-positive organisms in the tissues by floating paraffin sections on the warmed aqueous solution of methylene-blue or carbol-thionin gives beautiful results in most cases. It suffers from the drawback, however, that the histological picture is often difficult to interpret, and the preparations soon fade even when mounted in neutral balsam.

The two methods now to be described evade some of these difficulties and give preparations which are permanent.

1. *Iron-alum hæmatoxylin after mordanting with potassium bichromate and osmic acid.*

The method is a variant of that used to demonstrate mitochondria. Tissues fixed in formol-salt or formol-Müller are subjected to a further treatment with bichromate. Thin slices are placed in Müller's fluid for 2 to 7 days and then transferred to 2 per cent. osmic acid for two days more. They are then washed overnight in running water brought through alcohols and xylol and imbedded in paraffin. Sections not more than 5 microns thick, fixed on slides, are stained with $3\frac{1}{2}$ per cent. iron-alum solution at 50° C. for 15 minutes, followed

by ½ per cent. aqueous hæmatoxylin for an equal time at the same temperature. The sections should now be jet black. If such sections be decolorised in the ordinary way with iron-alum solution, mitochondria as well as bacteria retain the stain and the nuclei are decolorised. If, however, the sections are decolorised in 70 per cent. alcohol containing 0·5 per cent. hydrochloric acid, the mitochondria give up the lake while bacteria remain deeply stained. At the same time the details of nuclear structure are sharply coloured. The sections are now washed under the tap for 20 minutes, counterstained with any suitable stain, *e. g.*, v. Gieson's picric-acid fuchsin, and mounted in balsam. Fig. 1 (Pl. XII.), from a post-influenzal pneumonic lung, shows the appearance of such a preparation. It gives a good idea of the advantages of the method for the demonstration of B. influenzæ lying free and in phagocytes.

2. *van Gieson–Nile Blue Method.*

This method (an improvement on the one devised by Dr. A. H. Drew for demonstrating bacteria in tooth sections) is applicable to frozen or paraffin sections of material fixed in formol-salt solution. It apparently depends for success on the formation of a double salt of picric acid and Nile Blue in the tissues, and the relative insolubility in alcohol and clove oil of this compound when adsorbed by bacteria and certain cell elements. Tissues fixed in chromic mixtures (Zenker, Orth, &c.) are not suitable. The procedure is as follows :—

1. Picric acid–Acid fuchsin solution (v. Gieson) 1–3 minutes. Wash in distilled water.
2. 2 per cent. Nile Blue sulphate solution in distilled water 2–24 hours. Wash in changes of distilled water till the latter is tinted pale blue.
3. v. Gieson 1–5 minutes. Wash in changes of distilled water till the wash-water is pale yellow.
4. Dehydrate rapidly with absolute alcohol from a drop bottle.
5. Clear in xylol rapidly (not more than 1 minute).
6. Differentiate in clove oil—5 minutes to several hours. The longer period is necessary with thick frozen sections.
7. Wash off the clove oil with xylol and mount in Canada balsam.

In successful preparations nuclear chromatin and bacteria are stained a saturated transparent blue, mast cell granules nearly black, collagen red, keratin and erythrocytes orange yellow. Fibrin filaments stain either blue or orange red. It has been found that freshly prepared v. Gieson's solution is not so satisfactory as one which has been in use for some time. The fresh solution can be rapidly ripened by diluting with half its volume of distilled water and boiling for 2 or 3 minutes. Fig. 10 (Pl. X.), shows the appearance of a preparation stained by this method.

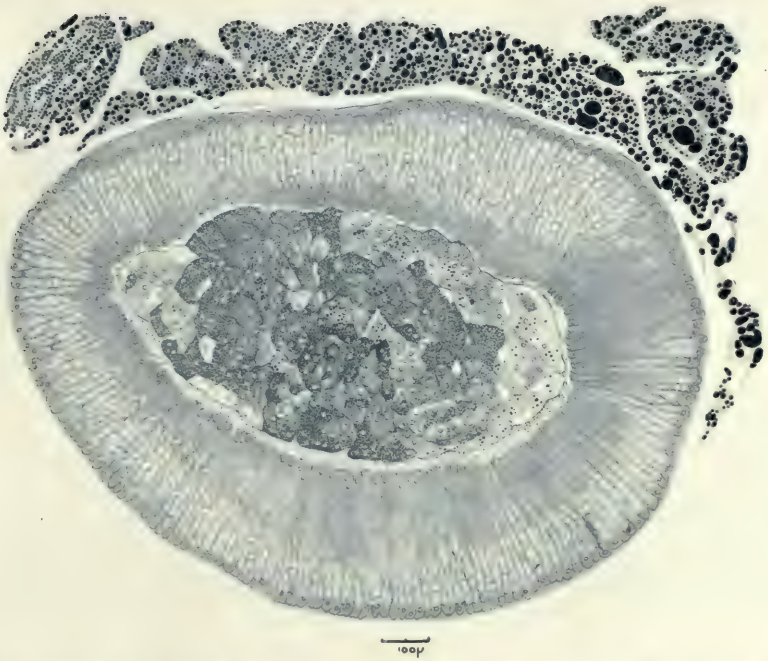


FIG. 1.—Suprarenal gland of normal mouse, fixed in osmic vapour. The lipoid granules of the cortex have been dissolved out in the Canada balsam, and are represented by vacuoles. The figure shows the sharp separation of medulla and cortex. In the medulla note two distinct appearances shown in the medullary cells by this method: cells uniformly filled with fine black granules and clear cells with larger black spheroids. The zona reticularis is narrow. The peri-adrenal adipose tissue has retained the osmic stained fat. Low power, × $\frac{60}{1}$

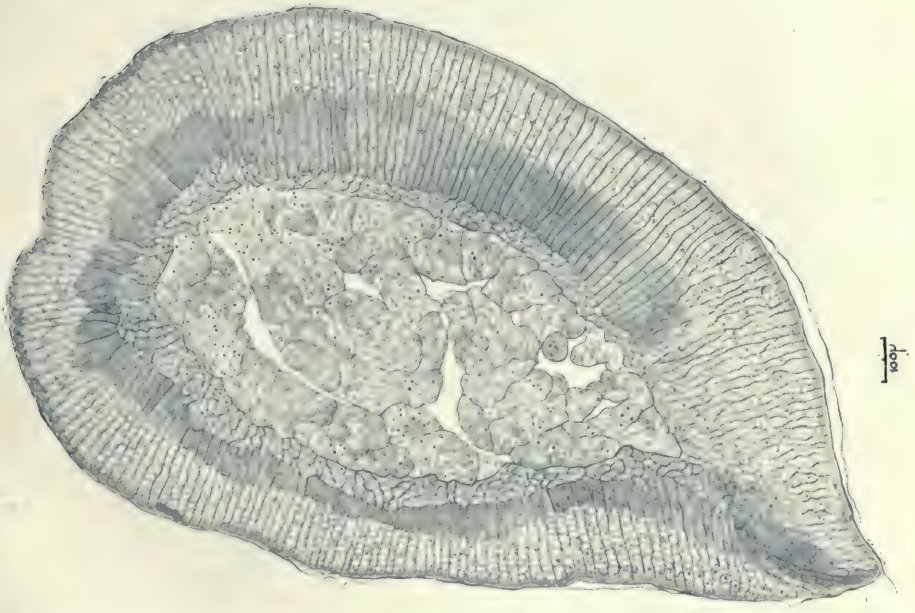
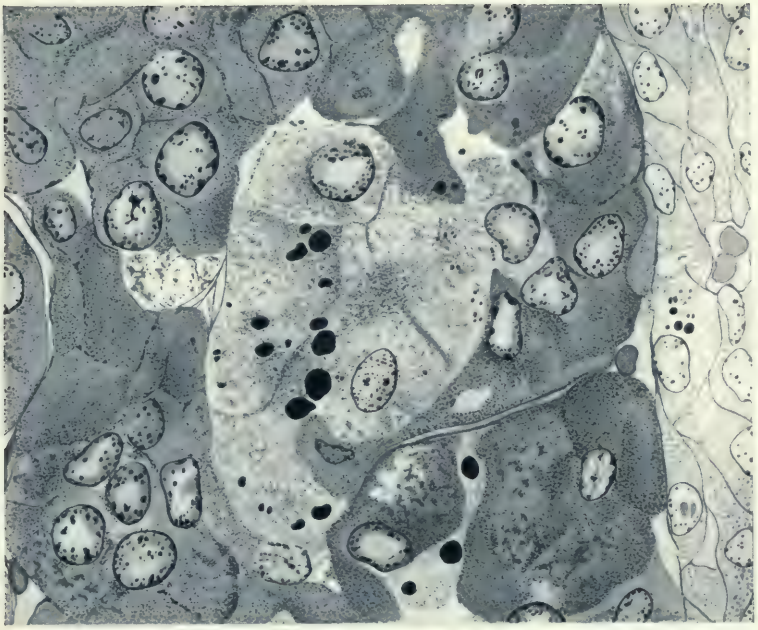
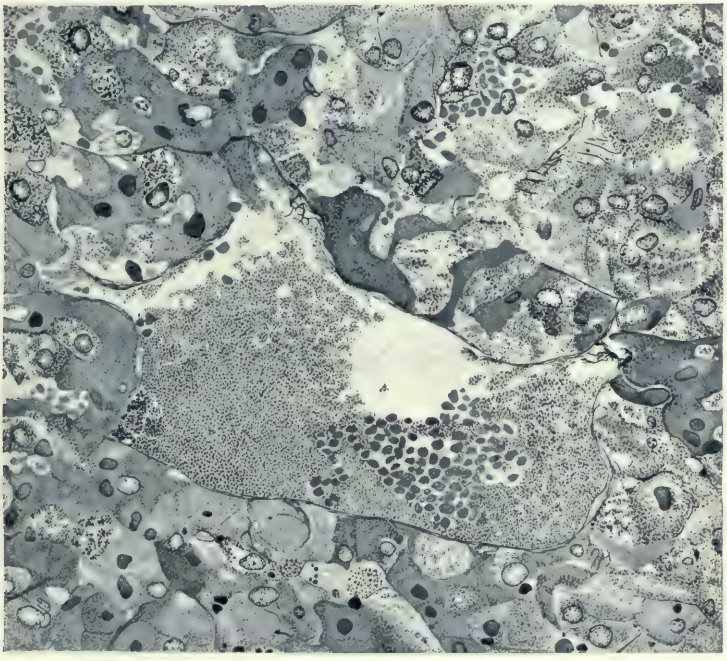


FIG. 2.—Exhausted suprarenal of a mouse dying as the result of exposure to wet and cold for 4 hours. The cortical lipoids are not obviously diminished, and are represented here also by vacuoles. The zona reticularis is swollen and broadened, and many of its cells contain small black globules. The medulla is completely exhausted, as shown by the absence of fine granules. A number of clear cells still contain black spheroids. Low power, × $\frac{60}{1}$



50μ

FIG. 3.—Suprarenal of a mouse which remained well after exposure to cold overnight for 9 consecutive nights and was then killed. Surface of medulla with a few layers of the swollen zona reticularis (on right). One of the cells of this zone contains a number of small black globules, which are not fats or lipoids. The figure shows a clear island in the medulla with black spheroids, surrounded by fully-loaded medullary cells. The appearance of the medullary cells in this figure is practically identical with that of a normal "resting" gland. High power, × $\frac{1100}{1}$



100μ

FIG. 4.—Suprarenal of a mouse which died 40 minutes after injection of 2·5 mg. T.H.N. The gland was swollen and congested. In the centre of the figure there is a vein nearly filled with fine osmic-blackened granules of adrenalin. Most of the cells are extensively vacuolated and contain adrenalin granules. These also occur in the intercellular spaces. Immediately bordering on the vein wall there are very darkly-stained "laked" cells with shrunken glazed nuclei. High power, × $\frac{480}{1}$

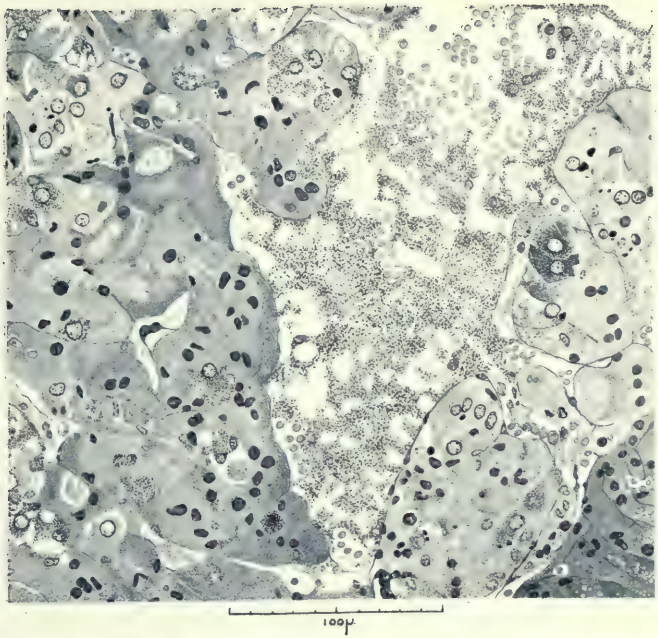


FIG. 5.—Suprarenal of a mouse which became collapsed after exposure to wet and cold for 1 hour: revived by application of warmth for 2 hours: again exposed to cold for 1 hour and then killed. A great number of the medullary cells are laked with shrunken glazed nuclei. The medullary cells with clear turgid nuclei are deeply vacuolated and have shed most of their adrenalin granules. There are many adrenalin granules in the lumen of the vein mixed with the plasma. High power, × $\frac{480}{1}$

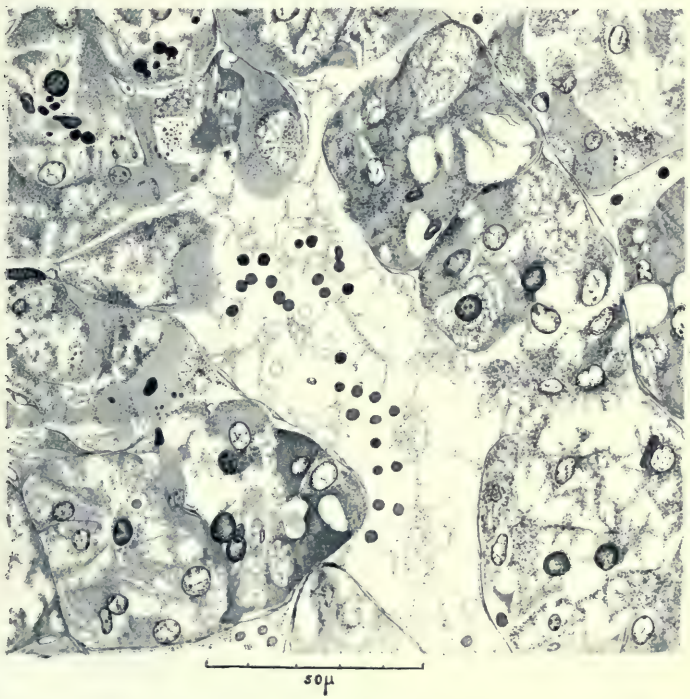


FIG. 6.—Suprarenal of a mouse which died 20 hours after injection of 0·5 c.c. Welch toxin (M.L.D. 0·3 c.c.) Examined 1 hour after death. Excessive vacuolation of majority of the medullary cells which only contain very few adrenalin granules and the central vein practically none. High power, × $\frac{560}{1}$

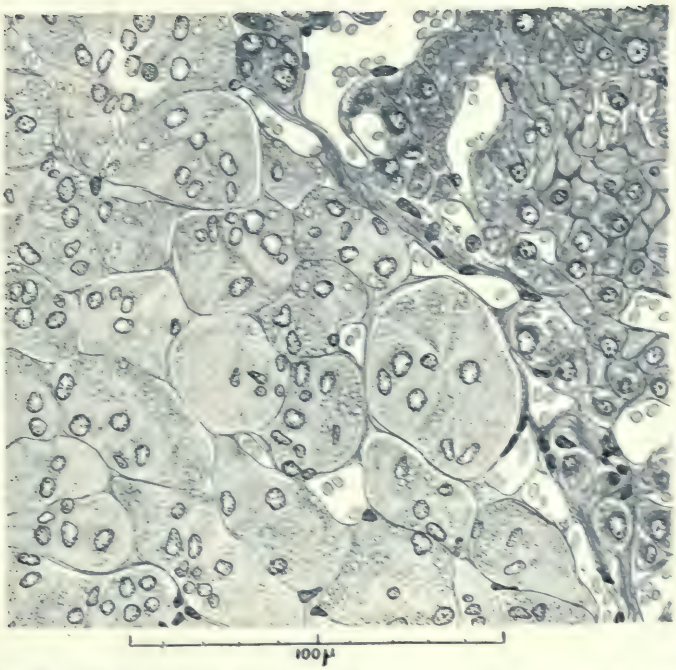


FIG. 7.—Normal suprarenal of rabbit. Zenker fixation. Junction of medulla and cortex. Cf. Fig. 8. High power, × $\frac{490}{1}$

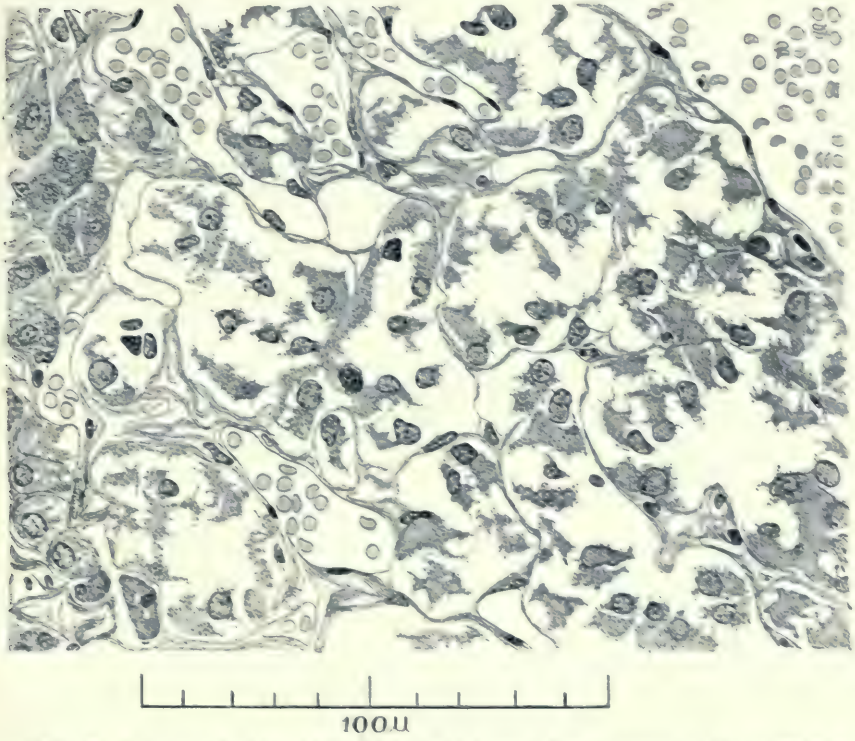


FIG. 8.—Suprarenal of a rabbit which died 2 hours after injection of 80 mg. T.H.N., hyperpyrexia 44° C. Zenker fixation. Junction of medulla and cortex. This figure gives the picture of an actively secreting gland obtained by ordinary fixatives and duplicates the appearances usually seen in human post-mortem material. High power, × $\frac{620}{1}$

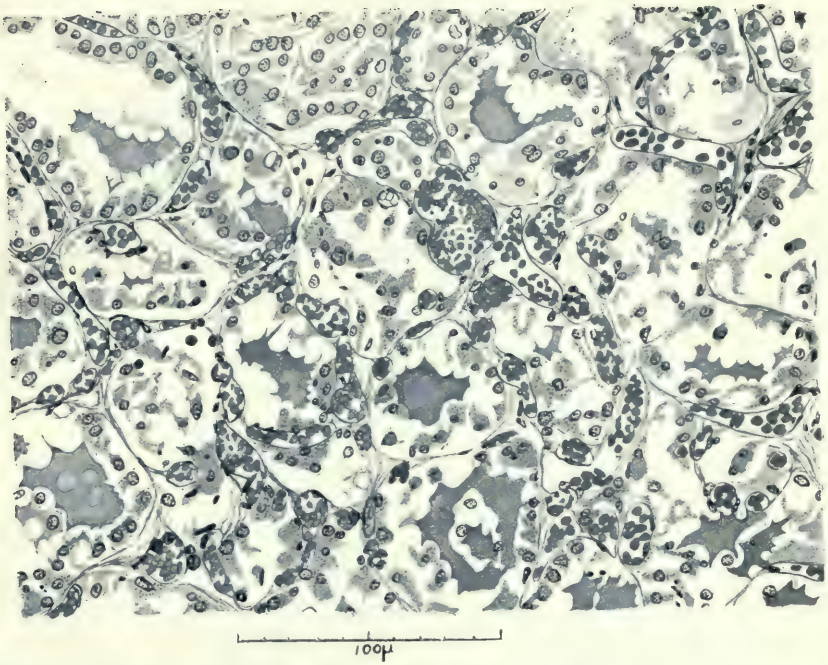


FIG. 9.—Thyroid gland of a rat which died with pulmonary hæmorrhages 2 hours after injection of 20 mg. T.H.N. The figure shows great vascular engorgement, desquamation of alveolar epithelium, and partial disappearance of the colloid. Zenker fixation. High power, × $\frac{350}{1}$

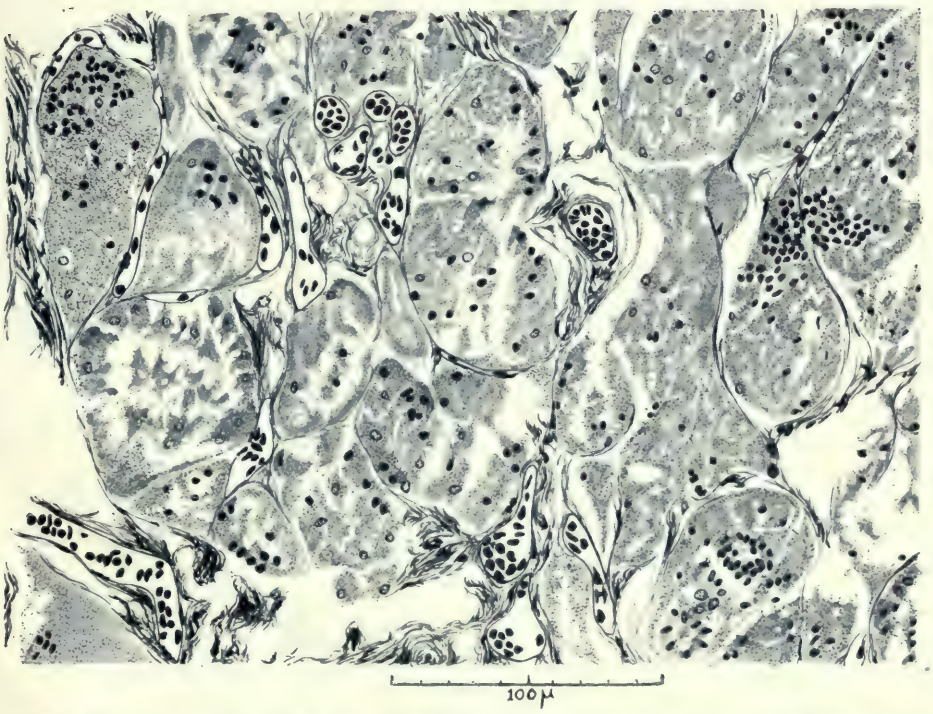


FIG. 10.—Human thyroid gland. Death from meningitis after prolonged severe pyrexia. The figure shows congestion and hæmorrhages into the alveoli, desquamation of epithelium and complete disappearance of colloid. Formol-Müller fixation followed by osmic acid. High power, × $\frac{360}{1}$

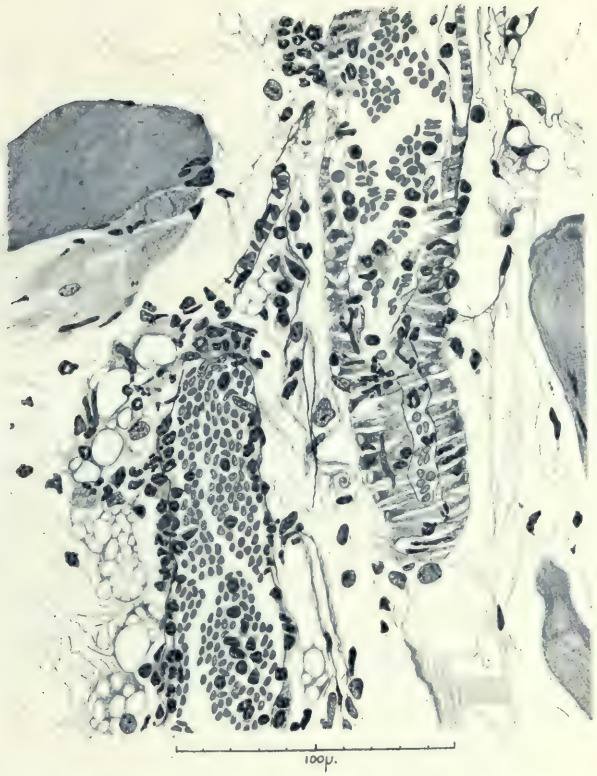


FIG. 2.—Early effect of injection of calcium nitrate. Mouse killed 6 hours after injection. Emigration of leucocytes and degeneration of muscle. × $\frac{370}{1}$

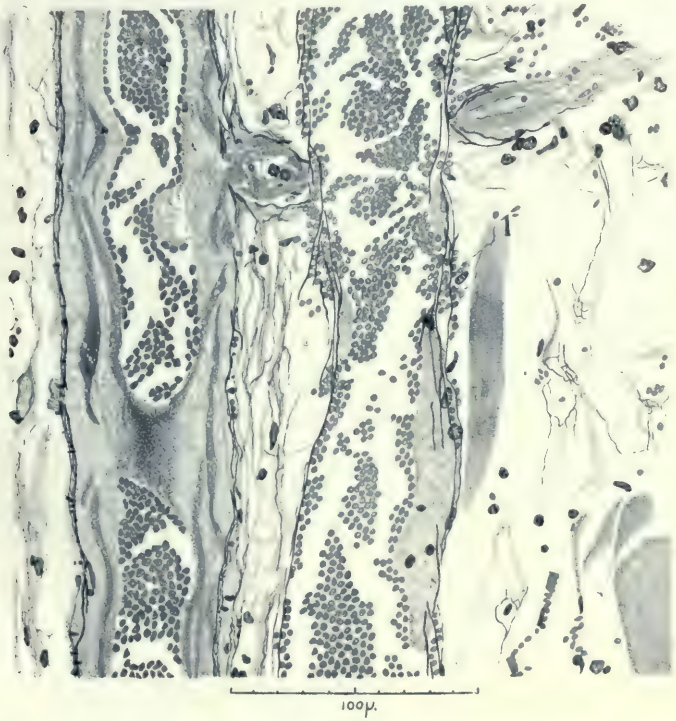


FIG. 3.—Fully developed calcium reaction—24 hours after injection of 10 mg. $Ca(NO_3)_2$. Thrombosis and loss of vascular endothelium. Degeneration of muscle fibres. × $\frac{330}{1}$

FIG. 5.—Calcium effect after 24 hours. Subcutaneous tissue. Dilated lymphatic filled with lymphocytes. Emigration of polymorphonuclear leucocytes.

FIG. 6.—Effect of strontium. 24 hours. Subcutaneous tissue. Engorged vessel and dilated lymphatic with clotted lymph.

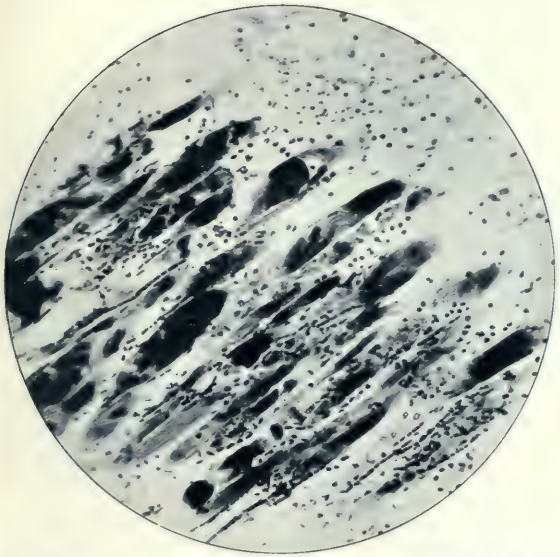


FIG. 4.—Calcium effect on abdominal wall after 24 hours. Degeneration of muscle, emigration of leucocytes.

FIG. 11.—Demonstrates phagocytosis of *B. welch* by leucocytes in an animal in which defence-ruptu has been produced by calcium salts. × $\frac{1280}{1}$

FIG. 7.—Effect of distilled water—24 hours, showing thrombosed blood-vessel and sterile abscess. Subcutaneous tissue.

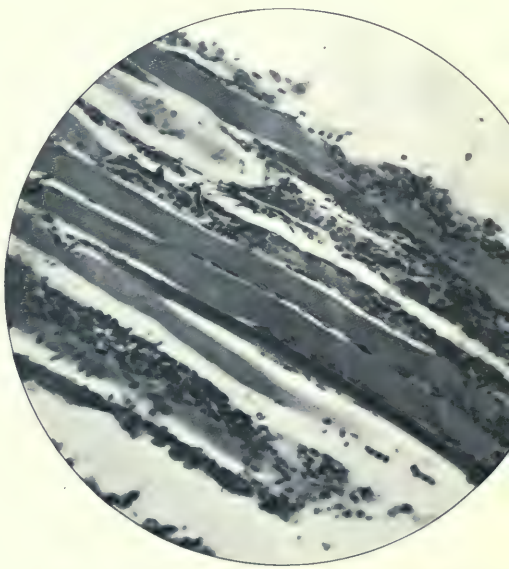
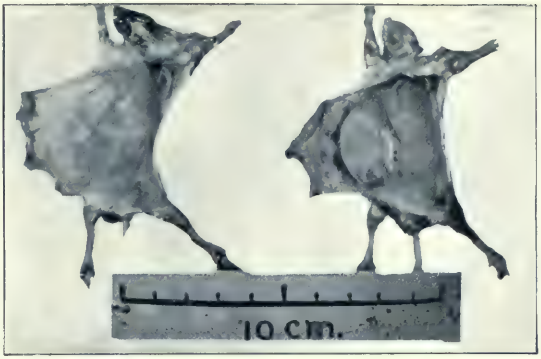


FIG. 8.—Magnesium effect. Abdominal wall aft 24 hours. Retention of transverse striation; emigr tion of lymphocytes.

24 hours.



Sodium (Control) Salt. Calcium Salt.

2 days.

Sodium Salt. Calcium Salt. Magnesium Salt.

3 days.

Sodium Salt. Calcium Salt. Magnesium Salt.

FIG. 9.—Defence-rupture in streptococcal infection by Calcium Salts.

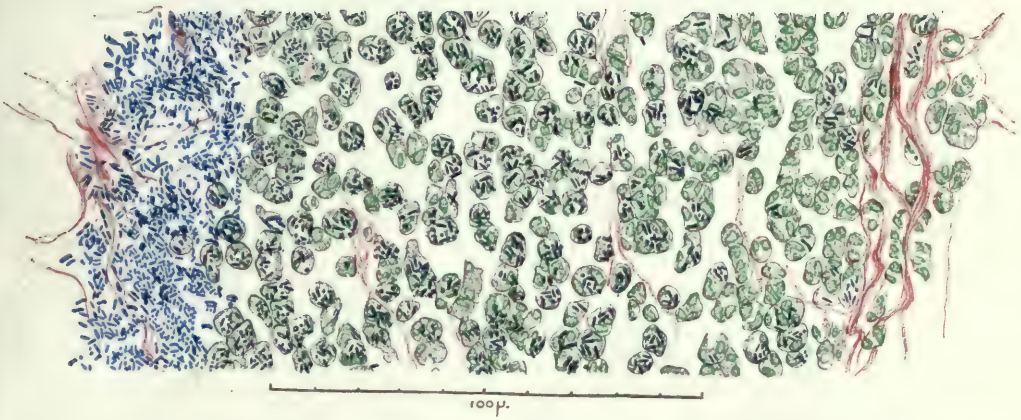


FIG. 10.—*B. welchii* and magnesium nitrate. Wall of circumscribed abscess consisting of leucocytes with ingested bacilli. Free bacilli on the left. × $\frac{570}{1}$

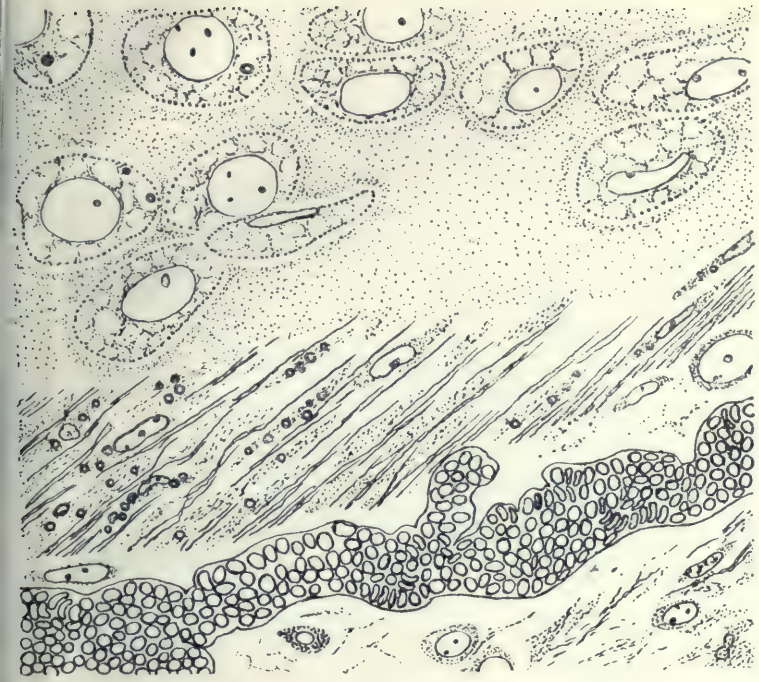
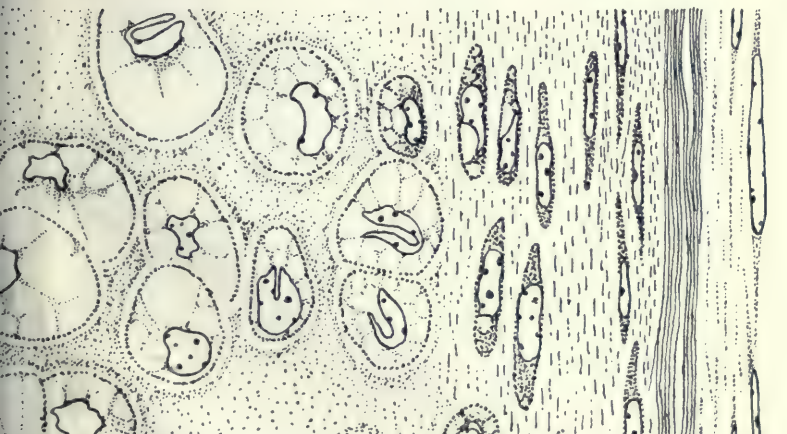


Fig. 1.—Mouse cartilage, homologous graft. Killed 2 days after transplantation. Fat droplets in cells of perichondrium, cartilage cells and matrix unaltered.



g. 2.—Mouse cartilage, homologous graft. Killed 4 weeks after transplantation. Nuclear furrows in cartilage cells indicating commencing amitosis.

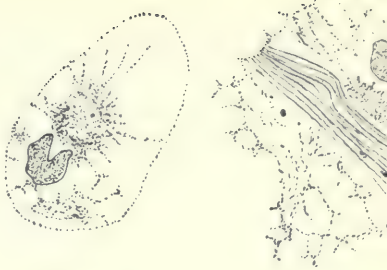


Fig. 3.

Fig. 4.

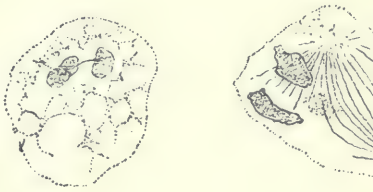


Fig. 5.

Fig. 6.

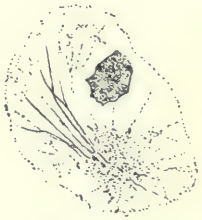


Fig. 7.

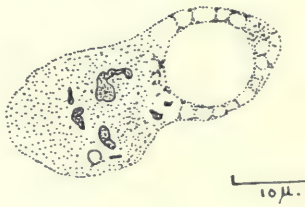


Fig. 8.

Fig. 9.

Figs. 3–9.—Human cartilage, autolo
Examined 6 months after tran
Figs. 3–7. Amitotic nuclear divisio
normal spindle and attraction fibres.
Early and late nuclear disintegratio
rhexis.

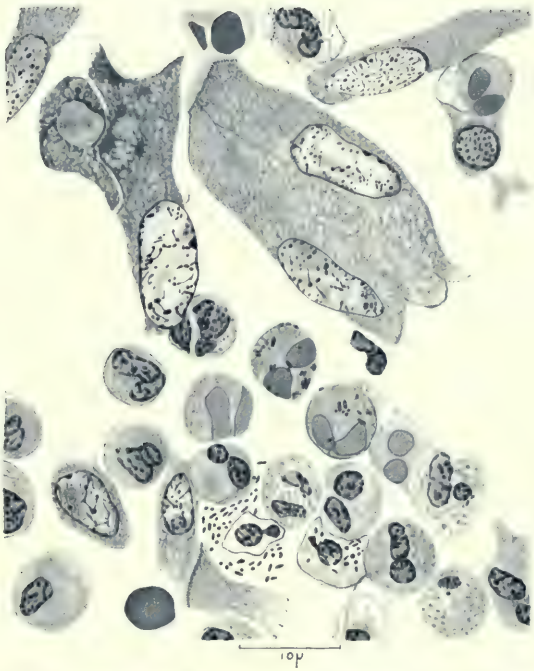
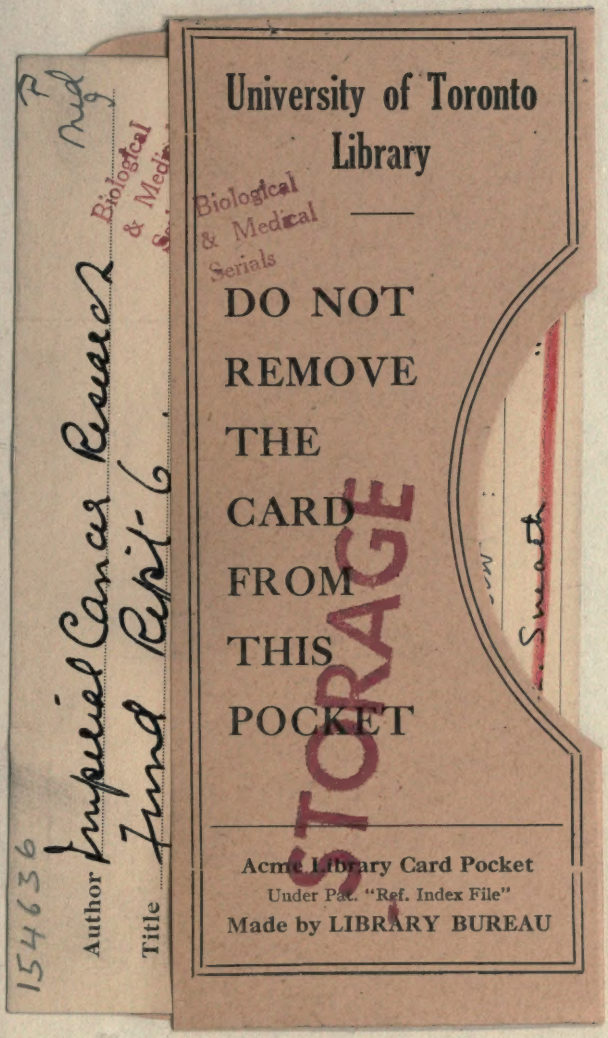


FIG. 1.—Free and phagocyted *B. influenzæ* in bronchial exudate from post-influenzal pneumonia. Iron-alum hæmatoxylin after potassium bichromate, osmic acid fixation. $\times \frac{1280}{1}$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